

**How DNA barcoding can be more effective in microalgae
identification: a case of cryptic diversity revelation in *Scenedesmus*
(Chlorophyceae)**

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Supplementary Table 1. List of specimens with the classification, collection locality, and voucher numbers. GenBank accession numbers are also given, some of which in bold were previously published.

| Strain designation | Collection locality | Species | GenBank accession number | | | |
|--------------------|------------------------------------|-----------------------|--------------------------|----------|----------|-------------|
| | | | <i>rbcL</i> | ITS | 16S | <i>tufA</i> |
| | | <i>Scenedesmus</i> | | | | |
| SM20-1 | Lake Hulunbeier, Neimeng, China | <i>S. deserticola</i> | KT777944 | KT778079 | KT778036 | KT778003 |
| SM20-3 | Lake Hulunbeier, Neimeng, China | <i>S. deserticola</i> | KT777945 | KT778078 | KT778037 | |
| SM20-4 | Soil from Shihezi, Xinjiang, China | <i>S. deserticola</i> | KT777950 | | KT778038 | |
| SM20_2 | Lake Hulunbeier, Neimeng, China | <i>S. deserticola</i> | | | KT778039 | KT778004 |
| SM2_4 | Lake Hulunbeier, Neimeng, China | <i>S. deserticola</i> | KT777960 | KT778060 | | |
| SM1_3 | Lake Hulunbeier, Neimeng, China | <i>S. deserticola</i> | KT777959 | KT778076 | KT778040 | KT777998 |
| SM16_2 | Lianyungang, China | <i>S. deserticola</i> | KT777955 | KT778067 | | |
| SM3_3 | Lake Hulunbeier, Neimeng, China | <i>S. deserticola</i> | KT777946 | KT778068 | KT778045 | KT778001 |
| SM5_3 | Lake Hulunbeier, Neimeng, China | <i>S. deserticola</i> | | KT778077 | | |
| SM3_2 | Soil from Shihezi, Xinjiang, China | <i>S. deserticola</i> | | KT778063 | KT778049 | KT778000 |
| SM3_4 | Lake Taihu, Jiangsu, China | <i>S. deserticola</i> | KT777949 | KT778073 | KT778054 | |
| SM4_3 | Lake Taihu, Jiangsu, China | <i>S. deserticola</i> | KT777965 | KT778059 | KT778046 | |
| SM5_4 | Lake Taihu, Jiangsu, China | <i>S. deserticola</i> | KT777966 | KT778057 | KT778057 | |
| SM3_1 | Lake Taihu, Jiangsu, China | <i>S. deserticola</i> | KT777967 | KT778062 | KT778053 | |
| SM2_1 | Lake Zixia, Jiangsu, China | <i>S. deserticola</i> | KT777968 | KT778072 | KT778050 | |
| SM5_2 | Lake Taihu, Jiangsu, China | <i>S. deserticola</i> | KT777969 | KT778121 | KT778056 | |
| SM5_1 | Lake Taihu, Jiangsu, China | <i>S. deserticola</i> | KT777970 | KT778058 | KT778055 | |
| SM4_1 | Lake Donghu, Wuhan, China | <i>S. deserticola</i> | KT777971 | KT778061 | KT778047 | KT777999 |
| SM1_2 | Lake Donghu, Wuhan, China | <i>S. deserticola</i> | KT777972 | KT778074 | KT778042 | KT777997 |
| SM4_2 | Lake Donghu, Wuhan, China | <i>S. deserticola</i> | KT777973 | KT778069 | KT778051 | KT778002 |

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|------------|------------------------------------|-----------------------|-----------------|----------|----------|-------------------|
| SM2_3 | Lake Donghu, Wuhan, China | <i>S. deserticola</i> | KT777974 | KT778064 | KT778048 | |
| SM1_4 | Lake Donghu, Wuhan, China | <i>S. deserticola</i> | KT777975 | KT778075 | KT778052 | KT777996 |
| 6JQ_2 | Lake Mochou, Nanjing, China | <i>S. deserticola</i> | KT777976 | KT778066 | KT778044 | |
| SM6_2 | Lake Mochou, Nanjing, China | <i>S. deserticola</i> | KT777963 | | | |
| SM4_4 | Lake Mochou, Nanjing, China | <i>S. deserticola</i> | KT777964 | KT778065 | KT818711 | |
| SM2_2 | Lake Mochou, Nanjing, China | <i>S. deserticola</i> | KT777985 | KT778071 | KT778043 | |
| SM1_1 | Lake Zixia, Jiangsu, China | <i>S. deserticola</i> | KT777986 | KT778070 | KT778041 | KT777995 |
| BCP-YPG | | <i>S. deserticola</i> | HQ246361 | | | HQ246381.1 |
| BCPEM2VF30 | | <i>S. deserticola</i> | HQ246358 | | | HQ246380.1 |
| | | <i>S. deserticola</i> | | | | HQ246379.1 |
| | | <i>S. deserticola</i> | | | | HQ246378.1 |
| | | <i>S. deserticola</i> | | | | HQ246377.1 |
| 1283_1 | Xiamen, Fujian, China | <i>S. spinosus</i> | KT777977 | KT778087 | | KT778006 |
| 1268_1 | Lake Taihu, Jiangsu, China | <i>S. spinosus</i> | | KT778091 | | KT778007 |
| 1268_2 | Lake Hulunbeier, Neimeng, China | <i>S. spinosus</i> | | KT778090 | | KT778009 |
| 1268_3 | Lake Boyang, China | <i>S. spinosus</i> | | KT778092 | | KT778008 |
| 1268_4 | Lake Taihu, China | <i>S. spinosus</i> | | KT778089 | | KT778010 |
| 1268_5 | Nanjing, China | <i>S. spinosus</i> | | KT778088 | | |
| 44_1 | Lake Hulunbeier, Neimeng, China | <i>S. quadricauda</i> | KT777982 | KT778084 | KT818697 | |
| 44_2 | Soil from Shihezi, Xinjiang, China | <i>S. quadricauda</i> | KT777980 | KT778085 | | |
| 44_4 | Lake Hulunbeier, Neimeng, China | <i>S. quadricauda</i> | KT777983 | KT778086 | KT818699 | KT778012 |
| 4_2 | Lake Hulunbeier, Neimeng, China | <i>S. quadricauda</i> | KT777981 | | | |
| 4_3 | Lake Mochou, Nanjing, China | <i>S. quadricauda</i> | KT777961 | | | |
| 40_3 | Lake Mochou, Nanjing, China | <i>S. quadricauda</i> | KT777979 | | KT818698 | |

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|--------|------------------------------------|-----------------------|-----------------|-------------------|-------------------|-----------------|
| | | <i>S. quadricauda</i> | AB084332 | | | |
| 76_1 | Donghu, Wuhan, China | <i>S. bijuga</i> | KT777978 | KT778082 | | KT778034 |
| 76_2 | Lake Taihu, Jiangsu, China | <i>S. bijuga</i> | | KT778080 | | KT778005 |
| 76_4 | Lake Zixia, Jiangsu, China | <i>S. bijuga</i> | | KT778081 | | |
| | | <i>S. rotundus</i> | HQ246351 | | | HQ246371 |
| | | <i>S. rotundus</i> | HQ246350 | | | HQ246372 |
| 959_1 | Lake Yueya, Nanjing, China | <i>S. dimorphus</i> | KT777956 | | | KT778033 |
| 959_2 | Lake Yueya, Nanjing, China | <i>S. dimorphus</i> | KT777958 | KT778101 | KT818705 | KT778013 |
| 959_3 | Lake Mochou, Nanjing, China | <i>S. dimorphus</i> | KT777984 | KT778102 | | KT778014 |
| 959_4 | Ningbo, Zhejiang, China | <i>S. dimorphus</i> | KT777957 | KT778103 | KT818706 | KT778024 |
| 959_5 | | | | | | KT778027 |
| 12_1 | Lake Donghu, Wuhan, China | <i>S. obliquus</i> | KT777952 | | KT818707 | KT778016 |
| 12_2 | River, Nanjing, China | <i>S. obliquus</i> | KT777954 | KT818720 | KT818703 | KT778026 |
| 12_3 | Lake Xuanwu, Nanjing, China | <i>S. obliquus</i> | KT777951 | KT778109 | | KT778015 |
| 12_4 | Lake Xuanwu, Nanjing, China | <i>S. obliquus</i> | KT777953 | KT778105 | KT818704 | KT778018 |
| 12_5 | Lake Hulunbeier, Neimeng, China | <i>S. obliquus</i> | | | | KT778017 |
| SM17_1 | Soil from Shihezi, Xinjiang, China | <i>S. obliquus</i> | KT777962 | KT778104 | KT818719 | |
| SM17_4 | Ningde, China | <i>S. obliquus</i> | KT777948 | KT778106 | KT818718 | |
| SM14_3 | Lake Hulunbeier, Neimeng, China | <i>S. obliquus</i> | | KT778117 | | |
| | | <i>S. obliquus</i> | | | EU073192.1 | |
| | | <i>S. obliquus</i> | | FR865721.1 | | |
| | | <i>S. obliquus</i> | | FR865719.1 | | |

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|--------|------------------------------------|----------------------------|-------------------|-------------------|-------------------|
| | | <i>S. obliquus</i> | FR865722.1 | | |
| | | <i>S. obliquus</i> | FR865726.1 | | |
| | | <i>S. obliquus</i> | FR865731.1 | | |
| | | <i>S. obliquus</i> | FR865738.1 | | |
| | | <i>S. obliquus</i> | FR865737.1 | | |
| | | <i>S. obliquus</i> | KJ676128.1 | | |
| | | <i>S. obliquus</i> | | | DQ396875.1 |
| | | <i>S. pectinatus</i> | FR865723.1 | | |
| | | <i>S. pectinatus</i> | FR865730.1 | | |
| | | <i>S. regularis</i> | FR865732.1 | | |
| SM9_1 | Lake Hulunbeier, Neimeng, China | <i>S. bajacalifornicus</i> | KT778119 | KT818700 | KT778021 |
| SM14_1 | Zhoushan, Zhejiang, China | <i>S. bajacalifornicus</i> | | | KT778030 |
| SM14_4 | Soil from Shihezi, Xinjiang, China | <i>S. bajacalifornicus</i> | KT778118 | | KT778029 |
| SM14_2 | Lake Hulunbeier, Neimeng, China | <i>S. bajacalifornicus</i> | KT778116 | | |
| SM9_5 | Lake Hulunbeier, Neimeng, China | <i>S. bajacalifornicus</i> | KT778115 | KT818702 | KT778028 |
| SM9_4 | Lake Hulunbeier, Neimeng, China | <i>S. bajacalifornicus</i> | KT778114 | | KT778031 |
| SM9_2 | Lianyungang, China | <i>S. bajacalifornicus</i> | KT778113 | | KT778019 |
| SM9_3 | Lake Hulunbeier, Neimeng, China | <i>S. bajacalifornicus</i> | | KT818701 | KT778035 |
| SM13_4 | Lake Hulunbeier, Neimeng, China | <i>S. bajacalifornicus</i> | KT778112 | KT818717 | KT778020 |
| SM13_3 | Soil from Shihezi, Xinjiang, China | <i>S. bajacalifornicus</i> | KT778111 | | KT778023 |
| SM13_1 | Lake Taihu, Jiangsu, China | <i>S. bajacalifornicus</i> | | | KT778025 |
| SM13_2 | Lake Xuanwu, Jiangsu, China | <i>S. bajacalifornicus</i> | KT778110 | KT818716 | KT778032 |
| | | <i>S. bajacalifornicus</i> | KF975592 | EU073189.1 | HQ246375.1 |

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|--------|---------------------------------|----------------------------|-----------------|----------|-------------------|
| | | <i>S. bajacalifornicus</i> | KF975602 | | HQ246374.1 |
| | | <i>S. bajacalifornicus</i> | HQ246355 | | HQ246376.1 |
| | | <i>S. bajacalifornicus</i> | HQ246354 | | |
| | | <i>S. bajacalifornicus</i> | HQ246357 | | |
| | | <i>S. bajacalifornicus</i> | HQ246353 | | |
| 1221_1 | River, Lianyungang, China | <i>S. acuminatus</i> | | KT778100 | KT818709 |
| 1221_4 | River, Nanjing, China | <i>S. acuminatus</i> | | KT778099 | KT818708 |
| 1271_3 | Lake Mochou, Nanjing, China | <i>S. acuminatus</i> | | | KT818710 |
| SM8_2 | Lake Hulunbeier, Neimeng, China | <i>S. sp</i> | KT777947 | KT778097 | |
| SM8_1 | Lake Taihu, Jiangsu, China | <i>S. sp</i> | | KT778095 | |
| SM8_3 | Lake Taihu, Jiangsu, China | <i>S. sp</i> | | KT778096 | |
| SM8_4 | Lake Xuanwu, Jiangsu, China | <i>S. sp</i> | | KT778098 | |
| SM15_1 | Lake Taihu, Jiangsu, China | <i>S. sp</i> | | KT778093 | |
| SM15_4 | Lake Donghu, Wuhan, China | <i>S. sp</i> | | KT778094 | KT778011 |
| | | <i>S. sp</i> | KF975604 | | |
| | | <i>S. sp</i> | | | EU073191.1 |
| | | <i>S. sp</i> | | | EU073193.1 |
| | | <i>S. sp</i> | | | EU073194.1 |
| | | <i>S. sp</i> | | | EU073195.1 |
| | | <i>S. sp</i> | | | AJ548893.1 |
| | | <i>S. sp</i> | | | AJ548894.1 |
| | | <i>S. sp</i> | | | KC994749.1 |
| | | <i>S. sp</i> | | | GQ920625.1 |
| | | <i>S. sp</i> | | | AF394206.1 |

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|----------------|------------------------------------|--------------------------|-------------------|----------|----------|-------------------|
| | | <i>S. sp</i> | | | | KC994774.1 |
| | | <i>S. sp</i> | | | | KC994812.1 |
| | | <i>S. sp</i> | | | | KC994840.1 |
| | | <i>S. sp</i> | | | | KC994867.1 |
| AKS-19 | | <i>S. sp</i> | KF975603.1 | | | |
| | | | | | | AJ548895.1 |
| Outgroup | | | | | | |
| | | <i>Chlorella</i> | | | | |
| 275_4 | Lake Xuanwu, Nanjing, China | <i>C. sorokiniana</i> | KT777992 | KT778083 | KT818713 | KR154271 |
| 275_1 | Qingdao, China | <i>C. sorokiniana</i> | KT777993 | KT778120 | KT818712 | KR154269 |
| 275_2 | Xiamen, Fujian, China | <i>C. sorokiniana</i> | KT777994 | KT778107 | | KR154270 |
| 5_4 | Lake Donghu, Wuhan, China | <i>C. ellipsoidea</i> | KT777991 | KT778122 | | |
| 5_3 | Soil from Shihezi, Xinjiang, China | <i>C. ellipsoidea</i> | KT777990 | | KT818715 | |
| 5_2 | Lake Mochou, Jiangsu, China | <i>C. ellipsoidea</i> | KT777989 | KM514842 | KT818714 | |
| 1_1 | River, Wuhan, China | <i>C. ellipsoidea</i> | KT777987 | | | |
| 2_2 | Lake Yueya, Nanjing, China | <i>C. ellipsoidea</i> | KT777988 | | | |
| CCAP 211/11M | | <i>C. emersonii</i> | | | | FR865657 |
| CCAP 211/15 | | <i>C. emersonii</i> | | | | FR865661 |
| IAM C-101 | | <i>C.pyrenoidosa</i> | | | | AJ242752.1 |
| | | <i>C.pyrenoidosa</i> | | | | JN887917.1 |
| | | <i>Desmodesmus</i> | | | | |
| Mary 8/18 T-1w | | <i>D.multivariabilis</i> | GU192431 | | | |

AKS-11

D. sp

KF975598

AKS-7

D. sp

KF975595.1

Supplementary Table S2. The mean interspecific divergences of *rbcL* sequences for *Scenedesmus* taxa (lower left: nucleotide divergences, upper right: standard error). The taxa name corresponds to the assignments in Figure 2.

| Taxa | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|----------|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Clade_10 | 1 | | 0.010 | 0.011 | 0.009 | 0.009 | 0.010 | 0.009 | 0.010 | 0.011 | 0.011 | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.012 | 0.012 | 0.019 |
| Clade_5 | 2 | 0.082 | | 0.005 | 0.008 | 0.010 | 0.010 | 0.009 | 0.009 | 0.010 | 0.010 | 0.012 | 0.011 | 0.012 | 0.012 | 0.012 | 0.012 | 0.011 | 0.019 |
| Clade_4 | 3 | 0.086 | 0.023 | | 0.009 | 0.010 | 0.010 | 0.009 | 0.008 | 0.009 | 0.009 | 0.012 | 0.012 | 0.011 | 0.011 | 0.012 | 0.011 | 0.010 | 0.019 |
| Clade_6 | 4 | 0.072 | 0.060 | 0.068 | | 0.010 | 0.011 | 0.010 | 0.011 | 0.011 | 0.011 | 0.013 | 0.012 | 0.012 | 0.012 | 0.013 | 0.011 | 0.011 | 0.019 |
| Clade_8 | 5 | 0.065 | 0.073 | 0.077 | 0.073 | | 0.002 | 0.004 | 0.008 | 0.010 | 0.010 | 0.011 | 0.011 | 0.012 | 0.012 | 0.012 | 0.012 | 0.011 | 0.019 |
| Clade_9 | 6 | 0.067 | 0.078 | 0.082 | 0.074 | 0.004 | | 0.005 | 0.008 | 0.010 | 0.010 | 0.011 | 0.011 | 0.012 | 0.012 | 0.012 | 0.012 | 0.011 | 0.019 |
| Clade_7 | 7 | 0.062 | 0.069 | 0.071 | 0.068 | 0.016 | 0.017 | | 0.007 | 0.009 | 0.009 | 0.012 | 0.010 | 0.012 | 0.011 | 0.011 | 0.011 | 0.010 | 0.018 |
| Clade_3 | 8 | 0.083 | 0.076 | 0.056 | 0.093 | 0.065 | 0.063 | 0.051 | | 0.006 | 0.005 | 0.012 | 0.011 | 0.011 | 0.012 | 0.012 | 0.011 | 0.010 | 0.018 |
| Clade_2 | 9 | 0.093 | 0.082 | 0.067 | 0.095 | 0.075 | 0.074 | 0.058 | 0.037 | | 0.007 | 0.013 | 0.009 | 0.011 | 0.010 | 0.010 | 0.010 | 0.010 | 0.017 |
| Clade_1 | 10 | 0.088 | 0.081 | 0.060 | 0.089 | 0.077 | 0.076 | 0.064 | 0.032 | 0.036 | | 0.013 | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.011 | 0.018 |
| Clade_11 | 11 | 0.108 | 0.094 | 0.093 | 0.115 | 0.093 | 0.093 | 0.096 | 0.106 | 0.104 | 0.106 | | 0.010 | 0.010 | 0.009 | 0.010 | 0.012 | 0.011 | 0.019 |
| Clade_18 | 12 | 0.105 | 0.089 | 0.089 | 0.107 | 0.089 | 0.092 | 0.079 | 0.089 | 0.071 | 0.089 | 0.067 | | 0.008 | 0.006 | 0.006 | 0.009 | 0.009 | 0.016 |
| Clade_12 | 13 | 0.101 | 0.098 | 0.087 | 0.108 | 0.096 | 0.099 | 0.095 | 0.092 | 0.090 | 0.091 | 0.068 | 0.046 | | 0.007 | 0.007 | 0.009 | 0.009 | 0.017 |
| Clade_17 | 14 | 0.105 | 0.095 | 0.087 | 0.105 | 0.095 | 0.098 | 0.086 | 0.094 | 0.077 | 0.086 | 0.067 | 0.030 | 0.034 | | 0.004 | 0.009 | 0.009 | 0.017 |
| Clade_16 | 15 | 0.104 | 0.098 | 0.093 | 0.108 | 0.096 | 0.099 | 0.087 | 0.092 | 0.078 | 0.092 | 0.068 | 0.028 | 0.037 | 0.012 | | 0.009 | 0.009 | 0.016 |
| Clade_15 | 16 | 0.098 | 0.094 | 0.087 | 0.095 | 0.099 | 0.099 | 0.087 | 0.088 | 0.081 | 0.089 | 0.090 | 0.055 | 0.058 | 0.058 | 0.057 | | 0.007 | 0.015 |
| Clade_14 | 17 | 0.102 | 0.089 | 0.078 | 0.090 | 0.090 | 0.092 | 0.078 | 0.076 | 0.069 | 0.070 | 0.083 | 0.055 | 0.059 | 0.055 | 0.054 | 0.035 | | 0.014 |
| Clade_13 | 18 | 0.228 | 0.226 | 0.216 | 0.223 | 0.228 | 0.230 | 0.214 | 0.213 | 0.203 | 0.216 | 0.205 | 0.168 | 0.178 | 0.173 | 0.166 | 0.156 | 0.136 | |

Supplementary Table S3. The mean interspecific divergences of ITS sequences for *Scenedesmus* taxa (lower left: nucleotide divergences, upper right: standard error). The taxa name corresponds to the assignments in Supplementary Figure 6.

| Taxa | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|----------|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Clade_1 | 1 | | 0.003 | 0.007 | 0.009 | 0.009 | 0.016 | 0.015 | 0.015 | 0.015 | 0.015 | 0.014 | 0.015 | 0.015 |
| Clade_2 | 2 | 0.011 | | 0.008 | 0.009 | 0.009 | 0.017 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 | 0.015 |
| Clade_3 | 3 | 0.046 | 0.053 | | 0.008 | 0.008 | 0.017 | 0.015 | 0.016 | 0.015 | 0.016 | 0.015 | 0.015 | 0.015 |
| Clade_4 | 4 | 0.073 | 0.083 | 0.070 | | 0.009 | 0.017 | 0.016 | 0.016 | 0.015 | 0.016 | 0.015 | 0.016 | 0.015 |
| Clade_5 | 5 | 0.066 | 0.071 | 0.058 | 0.084 | | 0.017 | 0.016 | 0.016 | 0.016 | 0.017 | 0.016 | 0.016 | 0.016 |
| Clade_8 | 6 | 0.189 | 0.192 | 0.208 | 0.217 | 0.201 | | 0.010 | 0.012 | 0.011 | 0.010 | 0.010 | 0.010 | 0.009 |
| Clade_6 | 7 | 0.162 | 0.165 | 0.165 | 0.193 | 0.179 | 0.093 | | 0.011 | 0.009 | 0.009 | 0.009 | 0.008 | 0.008 |
| Clade_12 | 8 | 0.171 | 0.171 | 0.179 | 0.199 | 0.190 | 0.118 | 0.096 | | 0.009 | 0.008 | 0.007 | 0.007 | 0.008 |
| Clade_13 | 9 | 0.166 | 0.169 | 0.170 | 0.189 | 0.182 | 0.099 | 0.070 | 0.059 | | 0.007 | 0.006 | 0.006 | 0.006 |
| Clade_11 | 10 | 0.159 | 0.163 | 0.169 | 0.184 | 0.179 | 0.088 | 0.068 | 0.049 | 0.038 | | 0.004 | 0.005 | 0.004 |
| Clade_10 | 11 | 0.156 | 0.160 | 0.161 | 0.185 | 0.174 | 0.089 | 0.067 | 0.045 | 0.032 | 0.016 | | 0.004 | 0.003 |
| Clade_14 | 12 | 0.157 | 0.160 | 0.161 | 0.188 | 0.175 | 0.087 | 0.064 | 0.044 | 0.031 | 0.018 | 0.013 | | 0.003 |
| Clade_9 | 13 | 0.160 | 0.164 | 0.166 | 0.186 | 0.180 | 0.083 | 0.064 | 0.050 | 0.033 | 0.019 | 0.011 | 0.012 | |

Supplementary Table S4. The mean interspecific divergences of 16S sequences for *Scenedesmus* taxa (lower left: nucleotide divergences, upper right: standard error). The taxa name corresponds to the assignments in Supplementary Figure 9.

| Taxa | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|----------|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Clade_1 | 1 | | 0.030 | 0.032 | 0.031 | 0.031 | 0.037 | 0.038 | 0.040 | 0.039 | 0.039 | 0.040 | 0.041 | 0.039 | 0.039 | 0.039 |
| Clade_2 | 2 | 0.237 | | 0.011 | 0.011 | 0.010 | 0.018 | 0.017 | 0.018 | 0.018 | 0.018 | 0.021 | 0.020 | 0.019 | 0.018 | 0.018 |
| Calde_3 | 3 | 0.269 | 0.048 | | 0.010 | 0.012 | 0.018 | 0.017 | 0.020 | 0.020 | 0.019 | 0.022 | 0.022 | 0.020 | 0.019 | 0.019 |
| Clade_4 | 4 | 0.258 | 0.046 | 0.036 | | 0.014 | 0.017 | 0.017 | 0.018 | 0.018 | 0.018 | 0.021 | 0.021 | 0.020 | 0.018 | 0.018 |
| Clade_5 | 5 | 0.262 | 0.037 | 0.051 | 0.064 | | 0.018 | 0.016 | 0.016 | 0.017 | 0.016 | 0.019 | 0.019 | 0.018 | 0.016 | 0.017 |
| Clade_6 | 6 | 0.318 | 0.106 | 0.108 | 0.103 | 0.103 | | 0.016 | 0.016 | 0.017 | 0.016 | 0.019 | 0.019 | 0.019 | 0.016 | 0.016 |
| Clade_14 | 7 | 0.325 | 0.095 | 0.101 | 0.102 | 0.083 | 0.086 | | 0.007 | 0.009 | 0.008 | 0.011 | 0.011 | 0.009 | 0.008 | 0.008 |
| Calde_13 | 8 | 0.344 | 0.102 | 0.117 | 0.111 | 0.080 | 0.083 | 0.020 | | 0.007 | 0.006 | 0.010 | 0.008 | 0.011 | 0.006 | 0.006 |
| Clade_12 | 9 | 0.338 | 0.100 | 0.120 | 0.115 | 0.088 | 0.097 | 0.034 | 0.022 | | 0.007 | 0.011 | 0.011 | 0.012 | 0.007 | 0.007 |
| Clade_11 | 10 | 0.338 | 0.102 | 0.107 | 0.102 | 0.086 | 0.080 | 0.023 | 0.014 | 0.017 | | 0.008 | 0.010 | 0.011 | 0.000 | 0.001 |
| Calde_10 | 11 | 0.351 | 0.133 | 0.138 | 0.133 | 0.116 | 0.110 | 0.050 | 0.042 | 0.045 | 0.027 | | 0.008 | 0.009 | 0.008 | 0.008 |
| Clade_9 | 12 | 0.350 | 0.128 | 0.143 | 0.138 | 0.105 | 0.108 | 0.043 | 0.023 | 0.045 | 0.037 | 0.031 | | 0.010 | 0.010 | 0.010 |
| Clade_15 | 13 | 0.334 | 0.118 | 0.123 | 0.125 | 0.105 | 0.109 | 0.026 | 0.040 | 0.055 | 0.043 | 0.039 | 0.036 | | 0.011 | 0.011 |
| Clade_8 | 14 | 0.338 | 0.102 | 0.107 | 0.102 | 0.086 | 0.080 | 0.023 | 0.014 | 0.017 | 0.000 | 0.027 | 0.037 | 0.043 | | 0.001 |
| Clade_7 | 15 | 0.339 | 0.103 | 0.108 | 0.103 | 0.087 | 0.081 | 0.024 | 0.016 | 0.019 | 0.002 | 0.029 | 0.039 | 0.045 | 0.002 | |

Supplementary Table S5. The mean interspecific divergences of *tufA* sequences for *Scenedesmus* taxa (lower left: nucleotide divergences, upper right: standard error). The taxa name corresponds to the assignments in Supplementary Figure 3.

| Taxa | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|----------|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Clade_1 | 1 | | 0.009 | 0.015 | 0.042 | 0.035 | 0.036 | 0.023 | 0.023 | 0.015 | 0.023 | 0.024 | 0.023 | 0.023 | 0.026 |
| Clade_2 | 2 | 0.024 | | 0.014 | 0.042 | 0.033 | 0.034 | 0.022 | 0.022 | 0.017 | 0.021 | 0.022 | 0.022 | 0.022 | 0.025 |
| Clade_3 | 3 | 0.068 | 0.057 | | 0.041 | 0.033 | 0.034 | 0.019 | 0.021 | 0.016 | 0.019 | 0.020 | 0.019 | 0.019 | 0.022 |
| Clade_4 | 4 | 0.363 | 0.368 | 0.357 | | 0.039 | 0.037 | 0.043 | 0.040 | 0.041 | 0.041 | 0.042 | 0.042 | 0.043 | 0.043 |
| Clade_5 | 5 | 0.270 | 0.255 | 0.250 | 0.325 | | 0.021 | 0.038 | 0.038 | 0.035 | 0.033 | 0.034 | 0.034 | 0.034 | 0.037 |
| Clade_6 | 6 | 0.300 | 0.278 | 0.291 | 0.320 | 0.139 | | 0.037 | 0.035 | 0.034 | 0.033 | 0.032 | 0.033 | 0.033 | 0.034 |
| Clade_7 | 7 | 0.147 | 0.139 | 0.111 | 0.380 | 0.295 | 0.312 | | 0.017 | 0.021 | 0.020 | 0.020 | 0.019 | 0.019 | 0.020 |
| Clade_8 | 8 | 0.139 | 0.127 | 0.131 | 0.368 | 0.300 | 0.295 | 0.083 | | 0.021 | 0.019 | 0.019 | 0.021 | 0.020 | 0.021 |
| Clade_9 | 9 | 0.064 | 0.083 | 0.076 | 0.374 | 0.280 | 0.296 | 0.119 | 0.127 | | 0.019 | 0.019 | 0.019 | 0.019 | 0.021 |
| Clade_10 | 10 | 0.139 | 0.119 | 0.107 | 0.352 | 0.259 | 0.279 | 0.110 | 0.107 | 0.095 | | 0.008 | 0.010 | 0.009 | 0.012 |
| Clade_11 | 11 | 0.148 | 0.127 | 0.115 | 0.364 | 0.265 | 0.271 | 0.115 | 0.107 | 0.103 | 0.021 | | 0.010 | 0.009 | 0.012 |
| Clade_13 | 12 | 0.135 | 0.131 | 0.103 | 0.357 | 0.265 | 0.287 | 0.107 | 0.123 | 0.099 | 0.031 | 0.032 | | 0.007 | 0.012 |
| Clade_14 | 13 | 0.147 | 0.127 | 0.107 | 0.371 | 0.269 | 0.282 | 0.108 | 0.118 | 0.109 | 0.030 | 0.029 | 0.019 | | 0.010 |
| Clade_12 | 14 | 0.168 | 0.147 | 0.127 | 0.368 | 0.290 | 0.290 | 0.111 | 0.119 | 0.115 | 0.043 | 0.039 | 0.042 | 0.039 | |

Supplementary Table S6. Species Delimitation Results of PID for *rbcL*. The species number corresponds to the P ID clades in Fig. 2.

| Species | Closest Species | Monophyletic? | Intra Dist | Inter Dist - Closest | Intra/Inter | P ID(Strict) | P ID(Liberal) | Av(MRCA-ti) | P(Randomly Distinct) | Clade Support | Rosenberg's P(AB) |
|---------|-----------------|---------------|------------|----------------------|-------------|-------------------------------|-------------------|-------------|----------------------|---------------|-------------------|
| 1 | 2 | yes | 2.667 | 3.667 | 0.73 | 0.30 (0.12, 0.49) | 0.60 (0.45, 0.74) | 1.6667 | 0.09 | NA | 0.17 |
| 2 | 3 | yes | 0.00E+00 | 3.304 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.17 |
| 3 | 2 | no | 2.569 | 3.304 | 0.78 | 0.73 (0.68, 0.79) | 0.92 (0.89, 0.95) | 1.3043 | NA | NA | NA |
| 4 | 5 | yes | 0.00E+00 | 4 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 2.65E-03 |
| 5 | 4 | yes | 2 | 4 | 0.5 | 0.60 (0.47, 0.72) | 0.87 (0.76, 0.97) | 1 | 1 | NA | 4.50E-08 |
| 6 | 4 | yes | 0.00E+00 | 4 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 1.68E-03 |
| 7 | 8 | yes | 0.00E+00 | 3 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.33 |
| 8 | 9 | yes | 0.00E+00 | 2 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 1 |
| 9 | 8 | yes | 0.00E+00 | 2 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 1 |
| 10 | 6 | yes | 0.00E+00 | 4 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 1.35E-03 |
| 11 | 10 | yes | 2 | 5 | 0.4 | 0.39 (0.23, 0.54) | 0.74 (0.58, 0.89) | 1 | 1 | NA | 4.10E-04 |
| 12 | 11 | yes | 2 | 5 | 0.4 | 0.60 (0.45, 0.74) | 0.86 (0.75, 0.97) | 1 | NA | 2.10E-05 | |
| 13 | 14 | yes | 2.5 | 3.75 | 0.67 | 0.42 (0.27, 0.56) | 0.73 (0.62, 0.84) | 1.75 | NA | 0.1 | |
| 14 | 13 | yes | 0.00E+00 | 3.75 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.1 |
| 15 | 14 | yes | 2 | 4 | 0.5 | 0.34 (0.18, 0.49) | 0.67 (0.52, 0.83) | 1 | 1 | NA | 0.02 |
| 16 | 17 | yes | 2 | 3 | 0.67 | 0.25 (0.09, 0.41) | 0.57 (0.41, 0.73) | 1 | NA | 0.33 | |
| 17 | 16 | yes | 0.00E+00 | 3 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.33 |
| 18 | 17 | yes | 2 | 4 | 0.5 | 0.60 (0.47, 0.72) | 0.87 (0.76, 0.97) | 1 | 1 | NA | 2.98E-03 |

Supplementary Table S7. Species Delimitation Results of PID for ITS. The species number corresponds to the P ID clades in Supplementary Fig. 6.

| Species | Closest Species | Monophyletic? | Intra Dist | Inter Dist - Closest | Intra/Inter | P ID(Strict) | P ID(Liberal) | Av(MRCA-tips) | P(Randomly Distinct) | Clade Support | Rosenberg's P(AB) |
|---------|-----------------|---------------|------------|----------------------|-------------|-------------------------------|-------------------|---------------|----------------------|---------------|-------------------|
| 1 | 2 | no | 2 | 3 | 0.67 | 0.79 (0.73, 0.84) | 0.94 (0.92, 0.97) | 1 | NA | NA | NA |
| 2 | 1 | yes | 2 | 3 | 0.67 | 0.25 (0.09, 0.41) | 0.57 (0.41, 0.73) | 1 | NA | 3.10E-04 | |
| 3 | 1 | yes | 2 | 5 | 0.4 | 0.52 (0.35, 0.70) | 0.78 (0.63, 0.93) | 1 | NA | 8.20E-04 | |
| 4 | 5 | yes | 2 | 4 | 0.5 | 0.46 (0.28, 0.64) | 0.72 (0.58, 0.87) | 1 | NA | 2.98E-03 | |
| 5 | 4 | yes | 2 | 4 | 0.5 | 0.60 (0.47, 0.72) | 0.87 (0.76, 0.97) | 1 | NA | 2.98E-03 | |
| 6 | 7 | yes | 2 | 3 | 0.67 | 0.25 (0.09, 0.41) | 0.57 (0.41, 0.73) | 1 | NA | 0.33 | |
| 7 | 6 | yes | 0.00E+00 | 3 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.33 |
| 8 | 7 | yes | 2 | 4 | 0.5 | 0.34 (0.18, 0.49) | 0.67 (0.52, 0.83) | 1 | NA | 0.05 | |
| 9 | 10 | yes | 3.278 | 5.778 | 0.57 | 0.70 (0.62, 0.79) | 0.91 (0.85, 0.96) | 1.7778 | NA | 1.60E-09 | |
| 10 | 11 | yes | 2 | 5 | 0.4 | 0.67 (0.54, 0.79) | 0.90 (0.80, 1.0) | 1 | NA | 2.70E-06 | |
| 11 | 10 | yes | 2 | 5 | 0.4 | 0.52 (0.35, 0.70) | 0.78 (0.63, 0.93) | 1 | NA | 1.40E-04 | |
| 12 | 13 | yes | 2 | 4 | 0.5 | 0.53 (0.38, 0.68) | 0.82 (0.71, 0.93) | 1 | NA | 0.03 | |
| 13 | 12 | yes | 2 | 4 | 0.5 | 0.34 (0.18, 0.49) | 0.67 (0.52, 0.83) | 1 | NA | 0.03 | |
| 14 | 11 | yes | 2 | 5 | 0.4 | 0.78 (0.69, 0.86) | 0.93 (0.88, 0.98) | 1 | 0.97 | NA | 2.80E-05 |

Supplementary Table S8. Species Delimitation Results of PID for *tufA*. The species number corresponds to the P ID clades in Supplementary Fig. 3.

| Species | Closest Species | Monophyletic? | Intra Dist | Inter Dist - Closest | Intra/Inter | P ID(Strict) | P ID(Liberal) | Av(MRCA-tips) | P(Randomly Distinct) | Clade Support | Rosenberg's P(AB) |
|---------|-----------------|---------------|------------|----------------------|-------------|-------------------------------|-------------------|---------------|----------------------|---------------|-------------------|
| 1 | 2 | yes | 4.75 | 7.375 | 0.64 | 0.60 (0.49, 0.70) | 0.86 (0.80, 0.92) | 4.375 | NA | 4.94E-03 | |
| 2 | 3 | yes | 2 | 5 | 0.4 | 0.39 (0.23, 0.54) | 0.74 (0.58, 0.89) | 1 | NA | 4.94E-03 | |
| 3 | 2 | yes | 2 | 5 | 0.4 | 0.39 (0.23, 0.54) | 0.74 (0.58, 0.89) | 1 | NA | 2.75E-03 | |
| 4 | 5 | yes | 0.00E+00 | 3 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.1 |
| 5 | 4 | yes | 0.00E+00 | 3 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.17 |
| 6 | 5 | yes | 2.667 | 3.667 | 0.73 | 0.30 (0.12, 0.49) | 0.60 (0.45, 0.74) | 1.6667 | NA | 0.17 | |
| 7 | 8 | yes | 0.00E+00 | 3 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.33 |
| 8 | 7 | yes | 2 | 3 | 0.67 | 0.25 (0.09, 0.41) | 0.57 (0.41, 0.73) | 1 | NA | 0.33 | |
| 9 | 7 | yes | 2 | 5 | 0.4 | 0.39 (0.23, 0.54) | 0.74 (0.58, 0.89) | 1 | 0.64 | NA | 4.10E-04 |
| 10 | 9 | yes | 3.167 | 7.25 | 0.44 | 0.57 (0.43, 0.72) | 0.85 (0.74, 0.96) | 2.25 | 0.97 | NA | 0.1 |
| 11 | 12 | yes | 4 | 6.333 | 0.63 | 0.51 (0.38, 0.64) | 0.81 (0.71, 0.91) | 3.3333 | 0.94 | NA | 5.60E-08 |
| 12 | 13 | yes | 0.00E+00 | 5.8 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 1.78E-03 |
| 13 | 12 | yes | 3.6 | 5.8 | 0.62 | 0.52 (0.39, 0.64) | 0.81 (0.71, 0.91) | 2.8 | 1 | NA | 1.10E-08 |
| 14 | 12 | yes | 6.514 | 9.133 | 0.71 | 0.71 (0.65, 0.78) | 0.91 (0.87, 0.96) | 6.1333 | 1 | NA | 4.30E-15 |

Supplementary Table S9. Species Delimitation Results of PID for 16S. The species number corresponds to the P ID clades in Supplementary Fig. 9.

| Species | Closest Species | Monophyletic? | Intra Dist | Inter Dist - Closest | Intra/Inter | P ID(Strict) | P ID(Liberal) | Av(MRCA-tips) | P(Randomly Distinct) | Clade Support | Rosenberg's P(AB) |
|---------|-----------------|---------------|------------|----------------------|-------------|-------------------------------|-------------------|---------------|----------------------|---------------|-------------------|
| 1 | 2 | yes | 2.5 | 3.917 | 0.64 | 0.44 (0.29, 0.58) | 0.75 (0.64, 0.86) | 1.75 | 1 | NA | 5.80E-08 |
| 2 | 1 | no | 2.294 | 3.917 | 0.59 | 0.82 (0.76, 0.87) | 0.95 (0.93, 0.98) | 1.1667 | NA | NA | NA |
| 3 | 4 | yes | 2.667 | 4.667 | 0.57 | 0.41 (0.23, 0.59) | 0.68 (0.54, 0.83) | 1.6667 | 0.87 | NA | 0.01 |
| 4 | 2 | yes | 2 | 4.167 | 0.48 | 0.54 (0.40, 0.69) | 0.83 (0.72, 0.94) | 1 | 0.54 | NA | 0.01 |
| 5 | 6 | yes | 2 | 4 | 0.5 | 0.34 (0.18, 0.49) | 0.67 (0.52, 0.83) | 1 | 0.54 | NA | 1.70E-04 |
| 6 | 7 | yes | 0.00E+00 | 3 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 2.85E-03 |
| 7 | 6 | no | 2 | 3 | 0.67 | 0.58 (0.47, 0.69) | 0.85 (0.79, 0.92) | 1 | NA | NA | NA |
| 8 | 11 | no | 2 | 2 | 1 | 0.08 (0.00E+00, 0.24) | 0.37 (0.21, 0.53) | 1 | NA | NA | NA |
| 9 | 10 | yes | 0.00E+00 | 2.5 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 1 |
| 10 | 9 | no | 3 | 2.5 | 1.2 | 0.00E+00 (0.00E+00, 0.14) | 0.24 (0.08, 0.40) | 1.5 | NA | NA | NA |
| 11 | 8 | yes | 0.00E+00 | 2 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.01 |
| 12 | 8 | yes | 2.333 | 3.833 | 0.61 | 0.52 (0.40, 0.65) | 0.82 (0.72, 0.92) | 1.8333 | NA | 6.30E-06 | |
| 13 | 8 | yes | 2 | 4 | 0.5 | 0.46 (0.28, 0.64) | 0.72 (0.58, 0.87) | 1 | 0.97 | NA | 0.02 |
| 14 | 15 | yes | 0.00E+00 | 3 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.33 |
| 15 | 14 | yes | 2 | 3 | 0.67 | 0.25 (0.09, 0.41) | 0.57 (0.41, 0.73) | 1 | NA | 0.33 | |

Supplementary Table 10. Combinations of diagnostic nucleotides for each of the 14 *Scenedesmus* taxa recovered in Supplementary Fig. 6 by CAOS. Nucleotide numbers refer to 28 selected positions on the ITS sequences.

| Species (Cryptic lineage number, Genbank number) | Positions | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| | 246 | 349 | 358 | 360 | 368 | 396 | 418 | 422 | 423 | 431 | 444 | 445 | 449 | 452 | 473 | 500 | 512 | 538 | 555 | 738 | 740 | 758 | 764 | 812 | 966 | 973 | 974 | 1208 |
| <i>Scenedesmus deserticola</i> I | C | A | C | C | - | - | T | C | T | C | G | A | C | T | - | G | C | T | - | C | T | G | C | C | G | T | C | T |
| <i>Scenedesmus deserticola</i> II | T | A | C | C | - | - | T | T | C | C | A | A | C | T | C | G | C | T | - | C | T | G | C | A | A | T | C | C |
| <i>Scenedesmus bijuga</i> | C | A | C | C | - | - | C | C | T | T | A | A | T | G | - | T | C | C | G | T | A | G | C | - | A | - | C | C |
| <i>Scenedesmus quadricauda</i> | C | A | C | C | - | - | C | C | T | C | A | C | C | G | - | G | C | T | G | T | T | G | C | A | G | T | A | C |
| <i>Scenedesmus spinosus</i> | C | A | C | C | - | - | C | T | T | C | A | A | C | G | - | A | C | T | G | T | A | G | T | - | A | - | C | C |
| <i>Scenedesmus</i> sp 2 | A | A | C | T | T | T | G | C | C | T | A | T | T | C | A | G | T | T | A | - | T | G | - | C | G | G | T | C |
| <i>Scenedesmus pectinatus</i> | A | A | C | T | C | A | G | C | T | T | - | - | T | C | A | G | A | C | A | T | G | G | - | G/T | A | T | C | C |
| <i>Scenedesmus regularis</i> | A | A | C | T | C | A | G | C | T | T | - | - | T | C | A | G | C | C | A | T | G | G | - | G | A | T | C | C |
| <i>Scenedesmus</i> sp 1 | A | T | A | C | T | C | T | C | C | T | - | T | T | T | A | G | C | C | A | C | C | A | A | C | A | T | C | G |
| <i>Scenedesmus acuminatus</i> | A | A | T | T | T | A | T | C | T | T | A | - | T | T | A | G | A | C | A | A | G | G | T | C | A | T | A | C |
| <i>Scenedesmus dimorphus</i> | A | A | T | T | A | A | T | C | T | T | - | - | - | T | A | G | C | T | A | T | T | A | T | T | A | T | C | G |
| <i>Scenedesmus obliquus</i> I,II,III | A | A | T | C | T | A | T | C | T | T | A | T | T | T | A | G | C | C | A | T | T | A | T | C | A | T | C | G |
| <i>Scenedesmus obliquus</i> IV | A | A | C | T | C | A | T | C | T | T | A | T | T | T | A | G | C | C | A | T | T | A | - | C | A | C | T | T |
| <i>Scenedesmus</i> sp 3 | A | A | C | T | T | G | T | C | T | T | A | G | T | T | A | G | C | C | A | T | T | A | T | C | A | T | C | G |

Supplementary Table S11. Combinations of diagnostic nucleotides for each of the 15 *Scenedesmus* taxa recovered by 16S in Supplementary Fig. 9 by CAOS analysis. Nucleotide numbers refer to 25 selected positions on the 16S sequences.

| Species (Cryptic lineage number and Genbank number) | Positions | | | | | | | | | | | | | | | | | | | | | | | | |
|---|-----------|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | 49 | 87 | 88 | 169 | 181 | 182 | 219 | 326 | 336 | 337 | 338 | 345 | 359 | 366 | 376 | 378 | 381 | 382 | 384 | 394 | 401 | 403 | 404 | 405 | 406 |
| <i>Scenedesmus deserticola</i> II | C | C | T | A | A | T | A | A | T | T | T | T | A | T | T | A | G | C | T | C | A | A | T | C | T |
| <i>Scenedesmus deserticola</i> I | C | C | T | C | C | T | A | G | G | A | A | C | G | G | C | G | A | T | C | T | C | A | T | C | A |
| <i>Scenedesmus quadricauda</i> | C | T | T | A | T | T | A | C | A | A | C | G | C | G | C | T | A | T | A | T | C | A | T | C | A |
| <i>Scenedesmus</i> sp (EU073191.EU073193.EU073194.EU073195.) | C | T | T | A | A | T | G | C | A | A | C | G | G | A | C | T | A | T | C | T | C | A | T | C | A |
| <i>Scenedesmus</i> sp (AJ548893.AJ548894.) | C | T | T | G | T | T | A | G | T | T | A | C | G | G | C | T | A | T | C | T | C | A | T | C | A |
| <i>Scenedesmus</i> sp (FJ608099.) | C | A | C | G | T | G | A | G | T | G | T | C | G | G | C | T | T | T | C | T | C | A | T | C | A |
| <i>Scenedesmus acuminatus</i> II | T | A | C | A | G | A | G | G | T | T | A | C | G | C | G | T | A | T | C | T | C | A | T | C | A |
| <i>Scenedesmus acuminatus</i> I | C | A | C | A | G | A | G | G | T | T | A | C | G | C | G | T | A | - | C | T | C | T | C | T | T |
| <i>Scenedesmus dimorphus</i> | C | A | C | G | G | A | G | G | T | T | A | C | G | C | G | G | A | T | C | T | C | A | T | C | A |
| <i>Scenedesmus obliquus</i> I- a | C | A | C | A | G | G | G | G | T | G | A | C | G | C | G | T | A | - | C | T | T | T | A | G | C |
| <i>Scenedesmus obliquus</i> I- b | C | A | C | A | G | G | G | G | T | G | A | C | G | C | G | T | A | - | C | A | A | T | C | A | T |
| <i>Scenedesmus obliquus</i> II | C | A | C | A | G | G | G | G | T | G | A | C | G | C | G | T | A | T | C | T | C | A | T | C | A |
| <i>Scenedesmus obliquus</i> III | C | A | C | G | G | A | G | G | T | T | A | C | G | C | G | G | A | - | C | T | T | T | T | G | C |
| <i>Scenedesmus bajacalifornicus</i> | G | G | C | A | G | A | G | G | T | G | A | C | G | C | G | T | A | T | C | T | C | A | T | C | A |
| <i>Scenedesmus obliquus</i> vs sp. | C | A | C | A | G | G | T | G | T | G | A | C | G | C | G | T | A | T | C | T | C | A | T | C | A |

Supplementary Table 12. Combinations of diagnostic nucleotides for each of the 14 *Scenedesmus* taxa recovered in Supplementary Fig. 3 by CAOS analysis. Nucleotide numbers refer to 39 selected positions on the *tufA* sequences.

| Species (Cryptic lineage number and Genbank number) | Positions | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | 137 | 173 | 195 | 197 | 198 | 200 | 212 | 215 | 216 | 227 | 251 | 254 | 258 | 263 | 272 | 287 | 290 | 317 | 323 | 329 | 335 | 347 | 350 | 353 | 368 | 371 | 375 | 389 | 393 | 402 | 404 | 408 | 423 | 429 | 430 | 434 | 452 | 472 | 473 |
| <i>Scenedesmus deserticola</i> I | T | A | G | A | A | C | T | C | A | C | T | T | G | C | A | A | C | T | A | C | A | A | T | A | T | T | T | A | G | G | A | T | T | G | T | T | C | C | T |
| <i>Scenedesmus deserticola</i> II | C | A | G | A | A | C | T | C | A | C | T | G | G | T | A | A | C | T | A | C | A | A | T | A | T | T | T | A | G | G | A | T | T | G | T | T | C | C | T |
| <i>Scenedesmus bijuga</i> | A | A | T | T | A | C | T | T | A | T | A | T | G | T | T | A | C | T | A | C | A | A | A | A | T | A | T | A | G | G | A | T | G | G | T | A | C | C | A |
| <i>Scenedesmus spinosus</i> I | T | T | G | T | C | A | C | T | C | T | T | T | G | T | T | T | A | C | T | T | T | A | C | A | T | G | G | T | G | G | T | A | A | A | C | T | T | G | A |
| <i>Scenedesmus spinosus</i> II | A | A | C | A | A | C | T | T | A | T | A | T | T | T | T | A | G | C | T | T | T | A | G | A | T | G | G | T | G | G | T | T | A | A | C | T | C | G | T |
| <i>Scenedesmus spinosus</i> III | A | A | G | A | A | A | T | T | A | T | A | T | T | C | T | A | G | C | T | T | C | T | T | A | T | A | G | T | G | A | T | T | A | A | C | T | T | G | C |
| <i>Scenedesmus</i> sp2 | A | A | T | A | G | T | A | T | A | C | A | A | A | C | A | A | A | T | A | C | T | G | A | A | A | A | T | A | G | G | T | T | T | G | T | A | C | C | A |
| <i>Scenedesmus rotundus</i> | A | C | A | A | G | T | A | C | A | T | A | T | A | C | A | A | C | T | A | C | T | A | C | T | A | A | T | A | A | G | T | T | T | G | T | C | T | C | A |
| <i>Scenedesmus quadricauda</i> | A | A | T | A | A | T | T | C | A | T | A | T | G | C | A | A | T | T | A | T | A | A | T | A | A | A | T | A | G | G | T | C | G | G | T | T | C | C | A |
| <i>Scenedesmus dimorphus</i> | A | A | A | A | A | T | T | C | C | T | A | T | G | T | A | T | T | T | A | T | T | A | A | A | A | A | T | A | G | G | T | T | G | G | T | T | C | C | A |
| <i>Scenedesmus obliquus</i> | A | A | A | A | A | T | T | C | A | T | A | T | G | T | A | T | T | T | A | T | T | T | A | A | A | A | T | A | G | A | T | T | G | G | T | T | C | C | A |
| <i>Scenedesmus deserticola</i> III | A | A | A | A | A | T | T | C | A | T | A | T | C | T | A | T | T | T | A | T | T | T | A | A | A | A | T | A | G | A | T | T | G | G | T | T | C | C | A |
| <i>Scenedesmus bajacalifornicus</i> | A | A | A | A | A | T | T | C | A | T | A | T | G | T | A | T | T | T | A | T | T | T | A | A | A | A | T | A | G | A | T | T | T | G | T | T | C | C | A |
| <i>Scenedesmus acuminatus</i> | G | G | A | A | A | T | T | C | A | T | A | T | G | T | A | T | T | T | A | T | T | G | A | A | A | A | T | A | G | A | T | T | G | G | T | T | T | C | A |

Supplementary Table 13. Species Delimitation Results of PID for *rbcL*+ITS+*tufA*+16S. The species number corresponds to the P ID clades in Fig. 3.

| Species | Closest Species | Monophyletic? | Intra Dist | Inter Dist - C | Intra/Inter | P ID(Strict) | P ID(Liberal) | Av(MRCA-tips) | P(Randomly) | Clade Support | Rosenberg's P(AB) |
|---------|-----------------|---------------|------------|----------------|-------------|---------------------|------------------|---------------|-------------|---------------|-------------------|
| 1 | 2 | yes | 0.005 | 0.033 | 0.16 | 0.87 (0.77, 0.98) | 0.96 (0.89, 1.0) | 0.0031 | NA | 4.94E-03 | |
| 2 | 1 | yes | 0.002 | 0.033 | 0.07 | 0.56 (0.41, 0.71) | 0.94 (0.79, 1.0) | 0.0011 | 0.06 | NA | 4.94E-03 |
| 3 | 1 | yes | 0.00E+00 | 0.064 | 0.00E+00 | 0.00E+00 (0.00E+00, | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.02 |
| 4 | 5 | yes | 7.44E-04 | 0.071 | 0.01 | 0.59 (0.44, 0.73) | 0.98 (0.83, 1.0) | 3.72E-04 | NA | 0.11 | |
| 5 | 4 | yes | 0.002 | 0.071 | 0.03 | 0.57 (0.42, 0.72) | 0.96 (0.81, 1.0) | 0.0011 | 0.11 | NA | 0.11 |
| 6 | 4 | yes | 0.002 | 0.229 | 0.01 | 0.59 (0.44, 0.74) | 0.98 (0.83, 1.0) | 0.0011 | 0.41 | NA | 9.10E-04 |

Supplementary Table 14. Species Delimitation Results of PID for *rbcL*+*tufA*. The species number corresponds to the P ID clades in Fig. 4.

| Species | Closest Species | Monophyletic? | Intra Dist | Inter Dist - | Intra/Inter | P ID(Strict) | P ID(Liberal) | Av(MRCA-tips) | P(Randomly Distinct) | Clade Supp | Rosenberg's P(AB) |
|---------|-----------------|---------------|------------|--------------|-------------|-------------------------------|-------------------|---------------|----------------------|------------|-------------------|
| 1 | 2 | yes | 0.019 | 0.039 | 0.49 | 0.69 (0.59, 0.80) | 0.89 (0.83, 0.96) | 0.0105 | 0.09 | NA | 0.04 |
| 2 | 1 | yes | 0.00E+00 | 0.039 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.04 |
| 3 | 4 | yes | 0.00E+00 | 0.137 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 1 |
| 4 | 2 | yes | 0.00E+00 | 0.077 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 1 |
| 5 | 2 | yes | 0.00E+00 | 0.08 | 0.00E+00 | 0.00E+00 (0.00E+00, 0.00E+00) | 0.96 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.01 |
| 6 | 5 | yes | 0.001 | 0.2 | 0.01 | 0.87 (0.72, 1.0) | 0.98 (0.87, 1.0) | 0.0012 | NA | 4.08E-03 | |

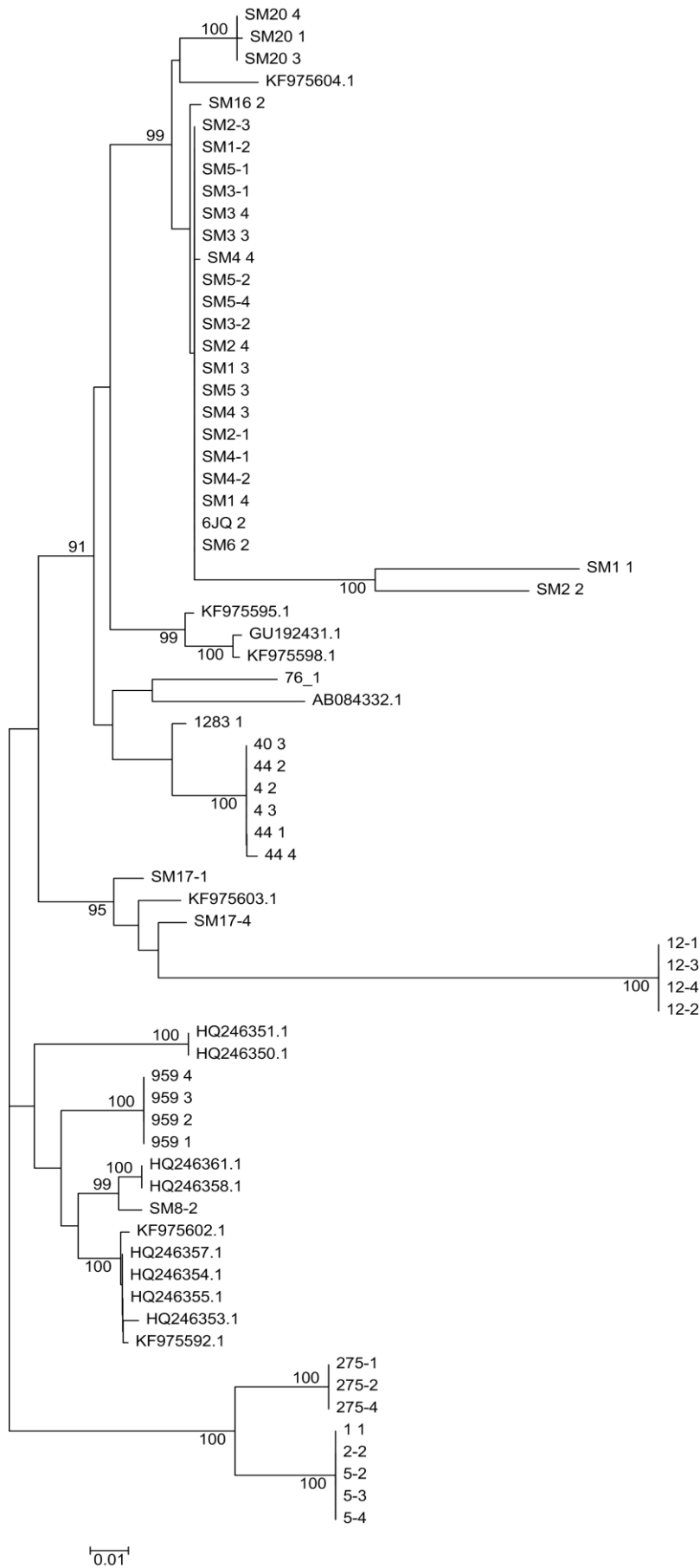
Supplementary Table 15. Species Delimitation Results of PID for ITS+16S. The species number corresponds to the P ID clades in Fig. 5.

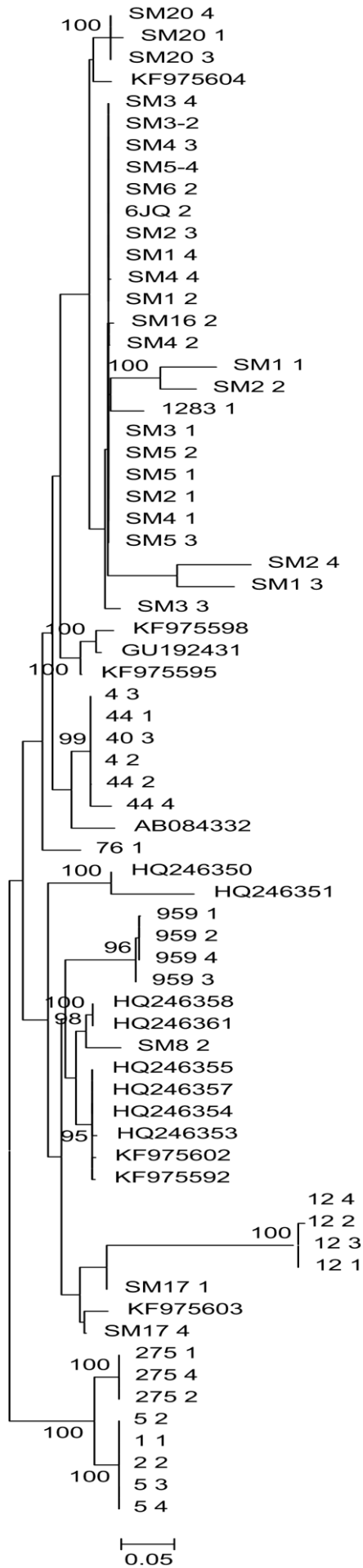
| Species | Closest Species | Monophyletic? | Intra Dist | Inter Dist - Closest | Intra/Inter | P ID(Strict) | P ID(Liberal) | Av(MRCA-tips) | P(Randomly Distinct) | Clade Support | Rosenberg's P(AB) |
|---------|-----------------|---------------|------------|----------------------|-------------|-------------------|-------------------|---------------|----------------------|---------------|-------------------|
| 1 | 3 | yes | 8.65E-04 | 0.06 | 0.01 | 0.98 (0.92, 1.0) | 1.00 (0.96, 1.0) | 4.45E-04 | NA | 9.10E-04 | |
| 2 | 1 | yes | 0.004 | 0.064 | 0.07 | 0.56 (0.41, 0.71) | 0.94 (0.79, 1.0) | 0.0022 | 0.93 | NA | 9.10E-04 |
| 3 | 1 | yes | 0.023 | 0.06 | 0.39 | 0.53 (0.35, 0.71) | 0.78 (0.64, 0.93) | 0.0119 | 1 | NA | 9.20E-05 |
| 4 | 8 | yes | 0.001 | 0.027 | 0.05 | 0.56 (0.41, 0.71) | 0.95 (0.80, 1.0) | 7.17E-04 | 0.99 | NA | 2.75E-03 |
| 5 | 6 | yes | 0.00E+00 | 0.007 | 0.00E+00 | 0.59 (0.44, 0.74) | 0.98 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.11 |
| 6 | 5 | yes | 0.004 | 0.007 | 0.6 | 0.29 (0.13, 0.44) | 0.61 (0.46, 0.77) | 0.0022 | NA | 0.11 | |
| 7 | 8 | yes | 0.00E+00 | 0.012 | 0.00E+00 | 0.59 (0.44, 0.74) | 0.98 (0.83, 1.0) | 0.00E+00 | NA | NA | 0.03 |
| 8 | 5 | yes | 0.002 | 0.011 | 0.2 | 0.73 (0.59, 0.88) | 0.93 (0.82, 1.0) | 0.0012 | 0.99 | NA | 0.03 |

Supplementary Table 16. Primer sequences and annealing temperatures used to amplify the different regions. Names of the forward (-F) and reverse (-R) primers pairs are indicated and also their references.

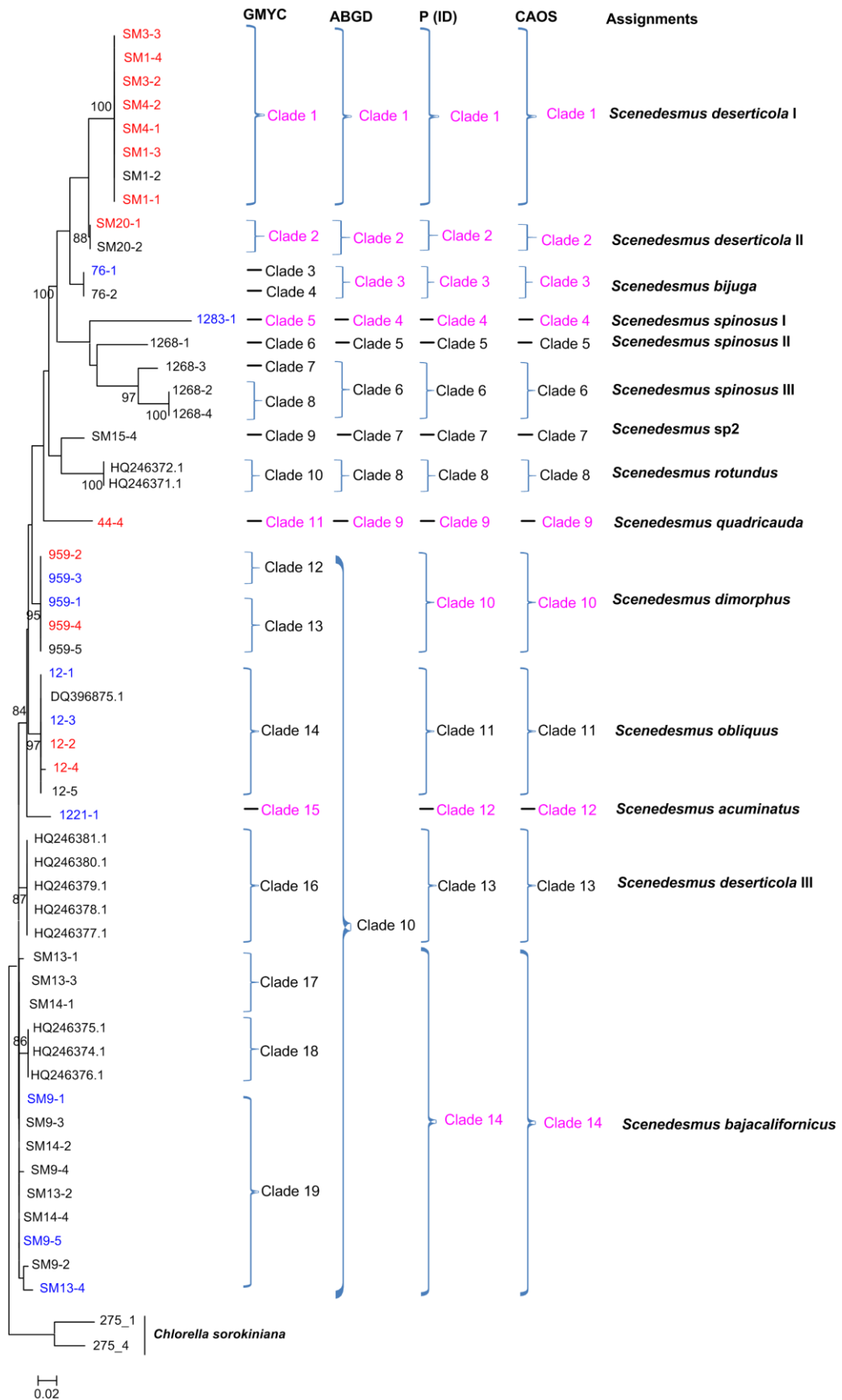
| Name | Sequence 5'–3' | Annealing temperature (°C) | Source |
|--------------------|-----------------------------------|-----------------------------------|-------------------|
| <i>rbcL</i> | | | |
| <i>rbcL</i> -F | ATG KCT CCA CAA ACT GAA ACT A | 49-51 | Sun et al. 2009 |
| <i>rbcL</i> -R | TTA AAG WGT ATC GAT WGT TTC GA | 49-51 | Sun et al. 2009 |
| <i>RcbLZ</i> -F | CAA CCA GGT GTT CCA SCT GAA G | 49-50 | This study |
| <i>RcbLZ</i> -R | CTA AAG CTG GCA TGT GCC ATA C | 49-50 | This study |
| 16S | | | |
| 359F-T | GGG GAA TTT TCC GCA ATG GG | 47-50 | Burja et al. 2001 |
| 781R(b) | GAC TAC AGG GGT ATC TAA TCC CTT T | 47-50 | Burja et al. 2001 |
| 16SZ-R | GGT ATC TWA TCC CTT TYG CT | 47-50 | This study |
| 16SZ-F | CGC AAT GGG CGA AAG CCT G | 47-50 | This study |
| ITS | | | |
| NS7m-F | GGC AAT AAC AGG TCT GT | 56 | Bock et al. 2011 |
| LR1850-R | CCT CAC GGT ACT TGT TC | 56 | Bock et al. 2011 |
| <i>tufA</i> | | | |
| <i>tufA</i> -F | TGA AAC AGA AMA WCG TCA TTA TGC | 47-53 | Famà et al. 2002 |
| <i>tufA</i> -R | CCT TCN CGA ATM GCR AAW CGC | 47-53 | Famà et al. 2002 |
| <i>tufA</i> -SF | TGG ATG GTG CWA TTY TWG | 47-50 | This study |
| <i>tufA</i> -SR | GGT TTT GCW AAA ACC ATW CCA CG | 47-50 | This study |

Supplementary Fig.
1. Neighbor joining
tree for the *rbcL*



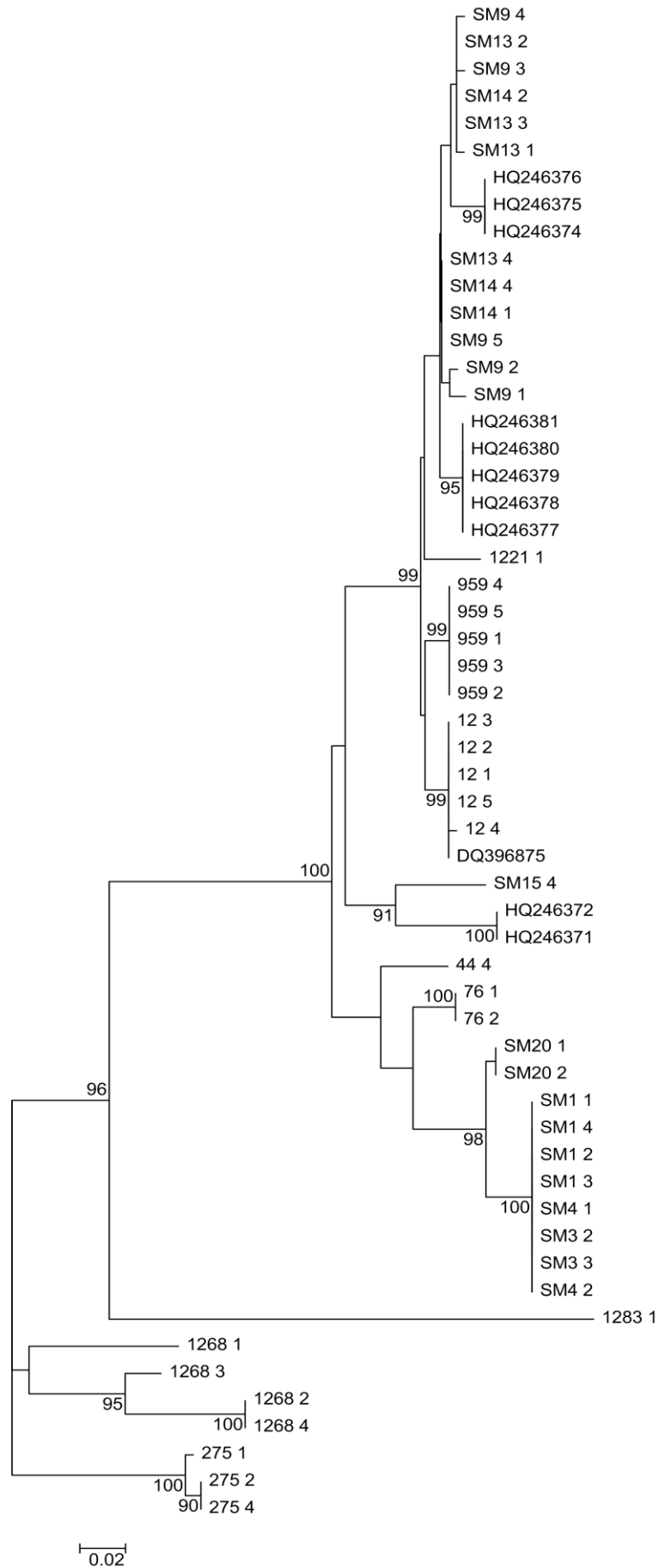


Supplementary Fig. 2.
Maximum likelihood tree
for the *rbcL* gene.

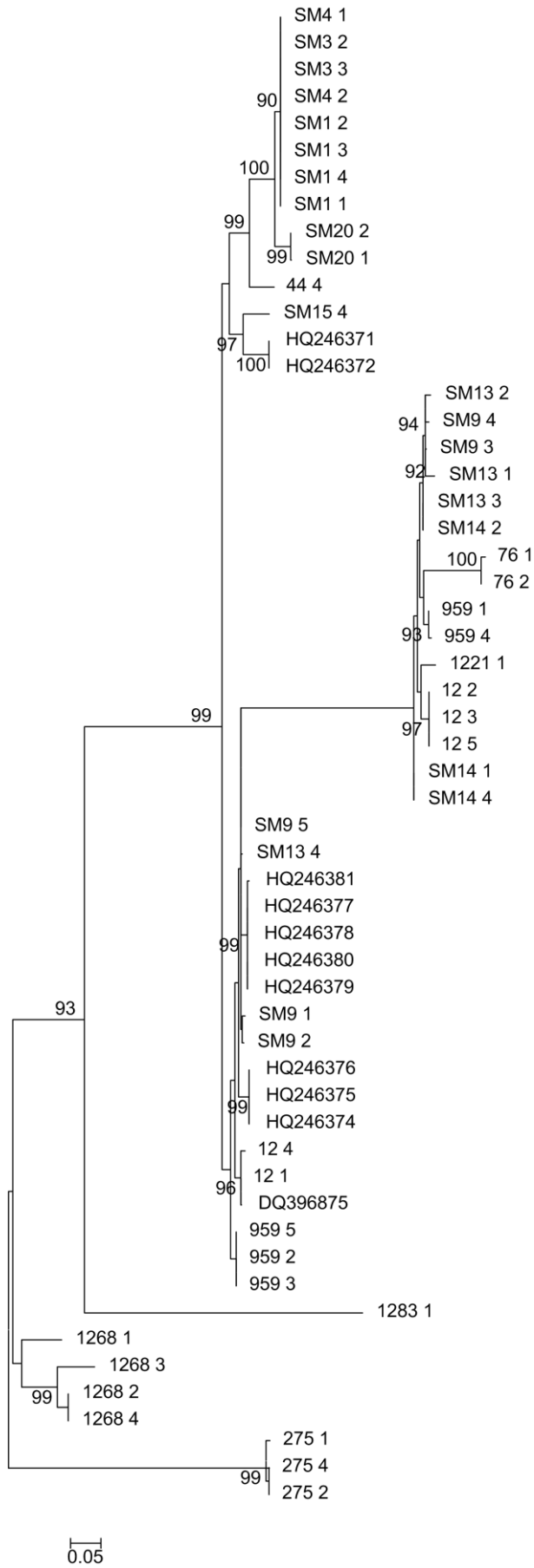


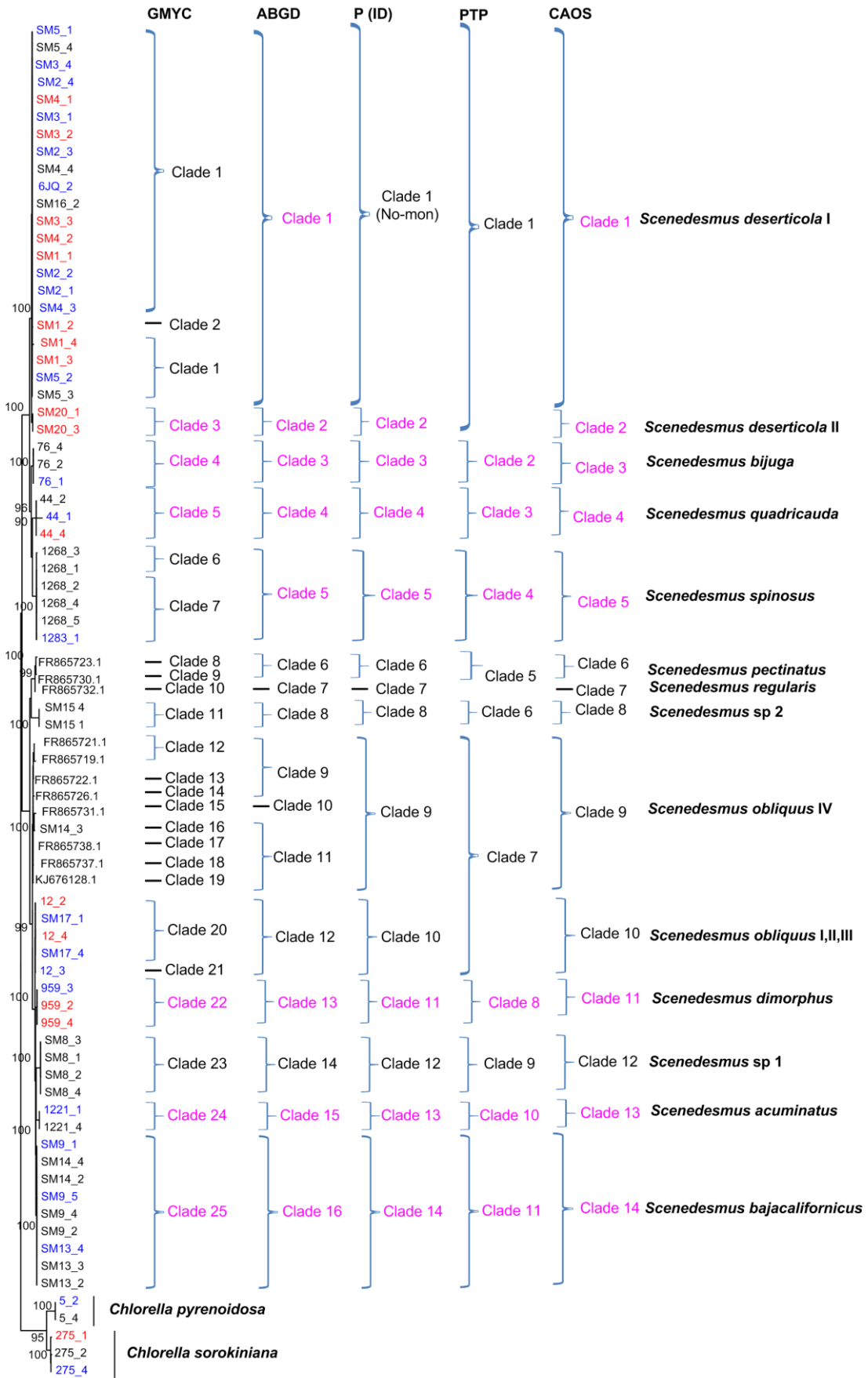
➡ Supplementary Fig. 3. Bayesian phylogenetic tree for the *tufA* gene. Vertical brace on the right indicate the clades detected by the tree-based GMYC, PID and the distance-based ABGD approach, the character-based CAOS and the taxa assignment. The clades highlighted in pink were also detected by *rbcL*, 16S and ITS gene loci. For samples colored in red, 16S, *rbcL* and ITS sequences were also available. For samples colored in blue, two of 16S, *rbcL* and ITS sequences were available.

Supplementary Fig. 4.
Neighbor joining tree for
the *tufA* gene.



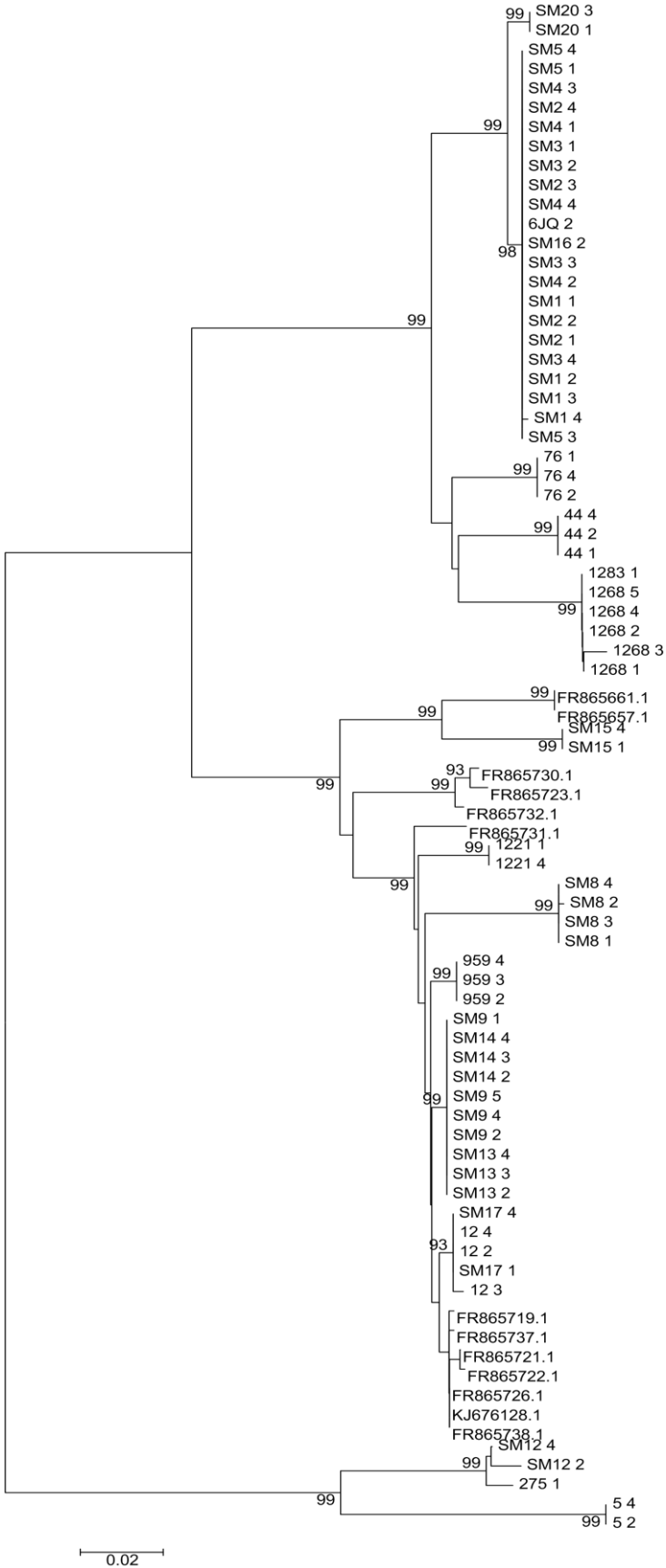
Supplementary Fig. 5.
Maximum likelihood tree
for the *tufA* gene.



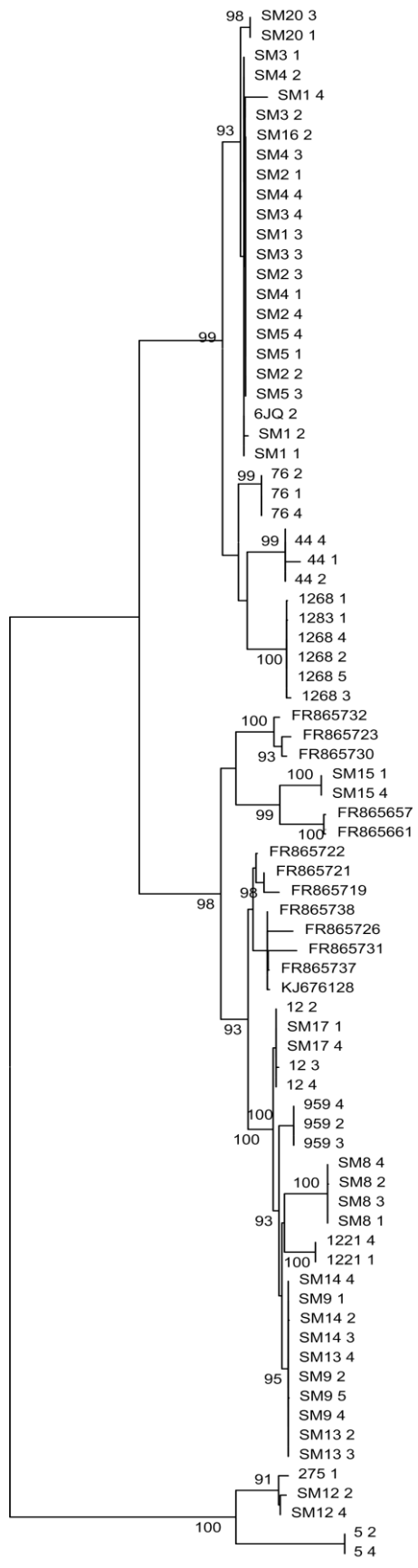


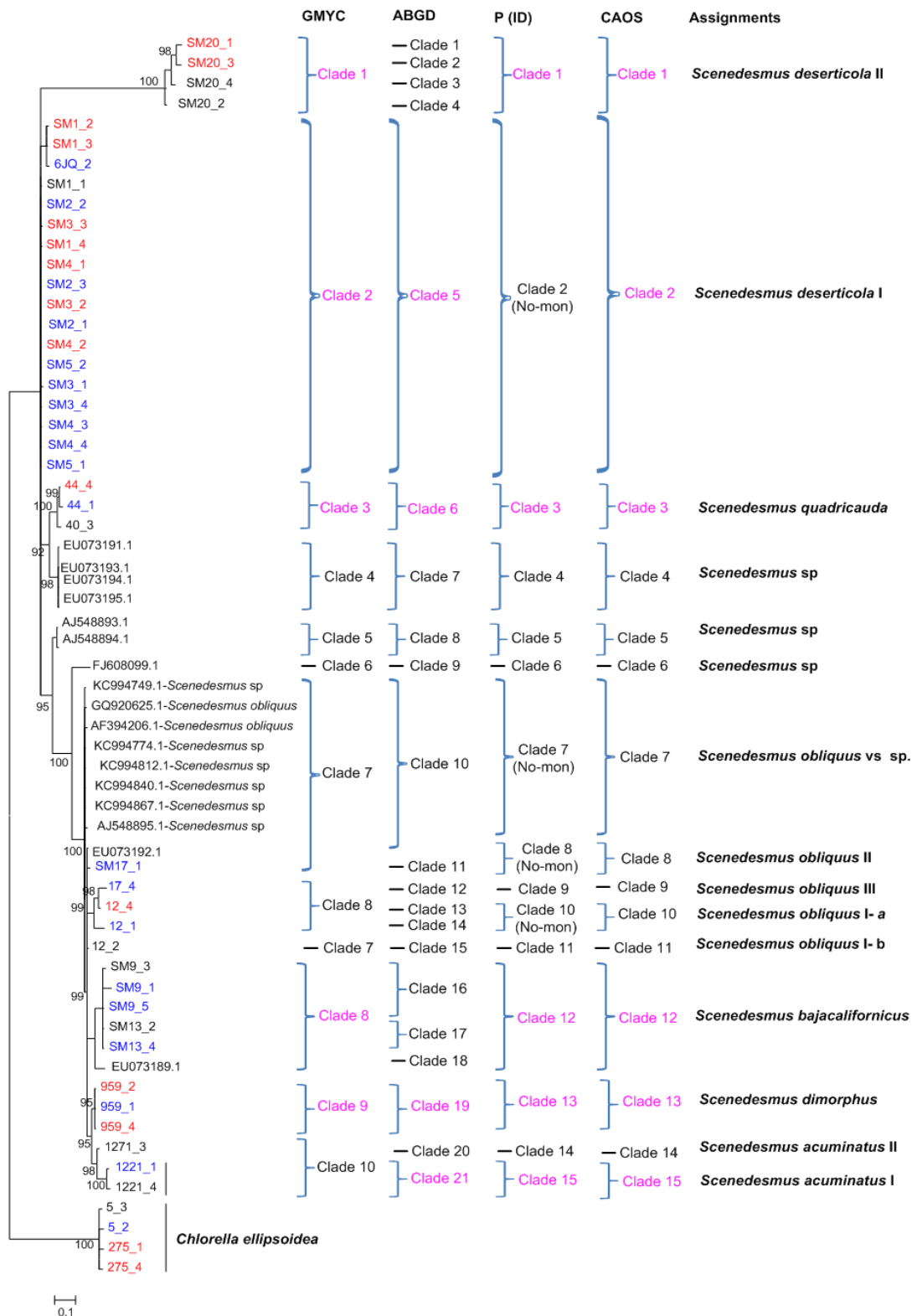
➡ Supplementary Fig. 6. Bayesian phylogenetic tree for the ITS gene. Vertical brace on the right indicate the clades detected by the tree-based GMYC, PID, PTP and the distance-based ABGD approach, the character-based CAOS and the taxal assignment. The clades highlighted in pink were also detected by *rbcL*, 16S and *tufA* gene loci. For samples colored in red, 16S, *rbcL* and *tufA* sequences were also available. For samples colored in blue, two of 16S, *rbcL* and *tufA* sequences were available.

Supplementary Fig. 7.
Neighbor joining tree for
the ITS gene.

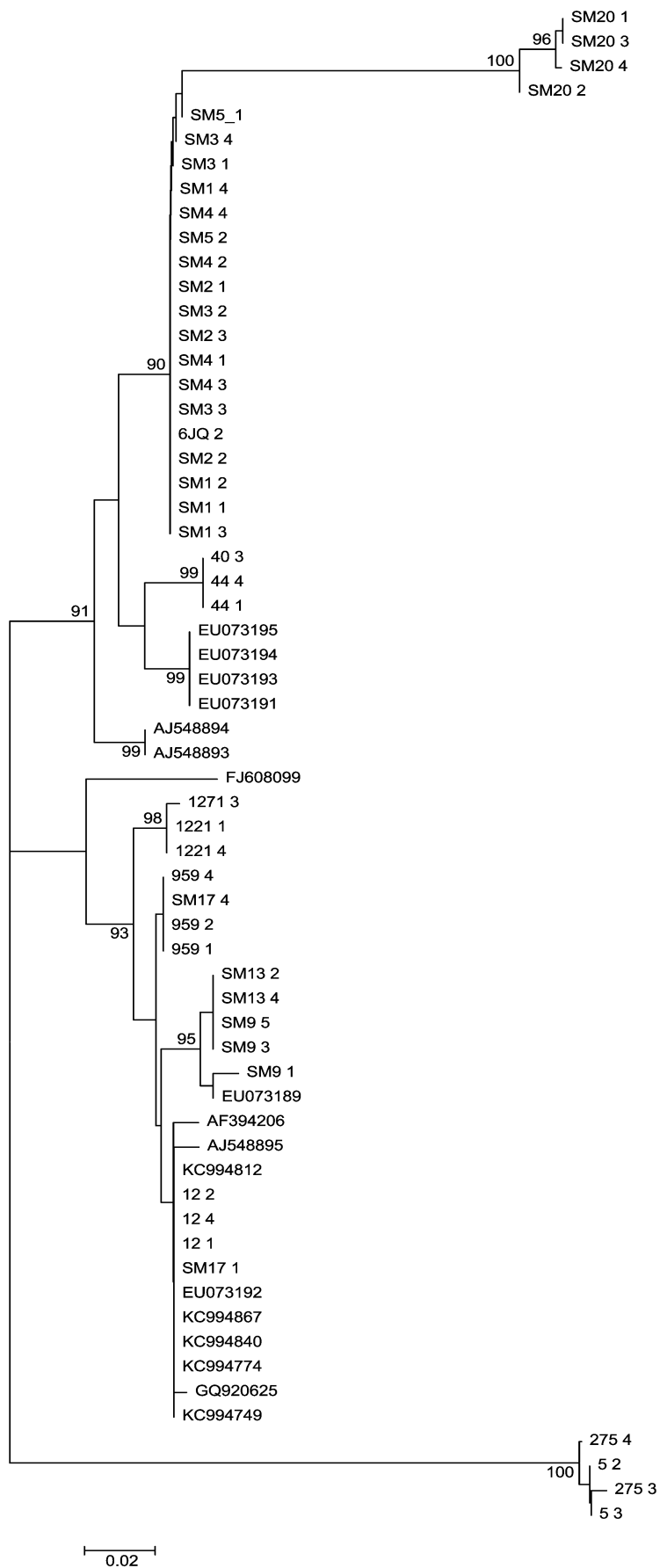


Supplementary Fig. 8.
Maximum likelihood tree
for the ITS gene.



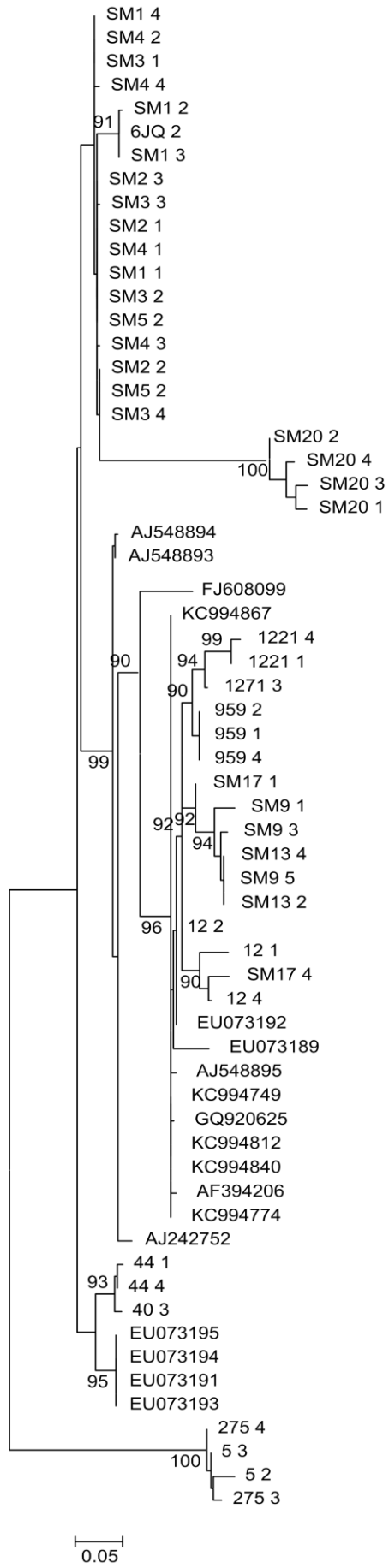


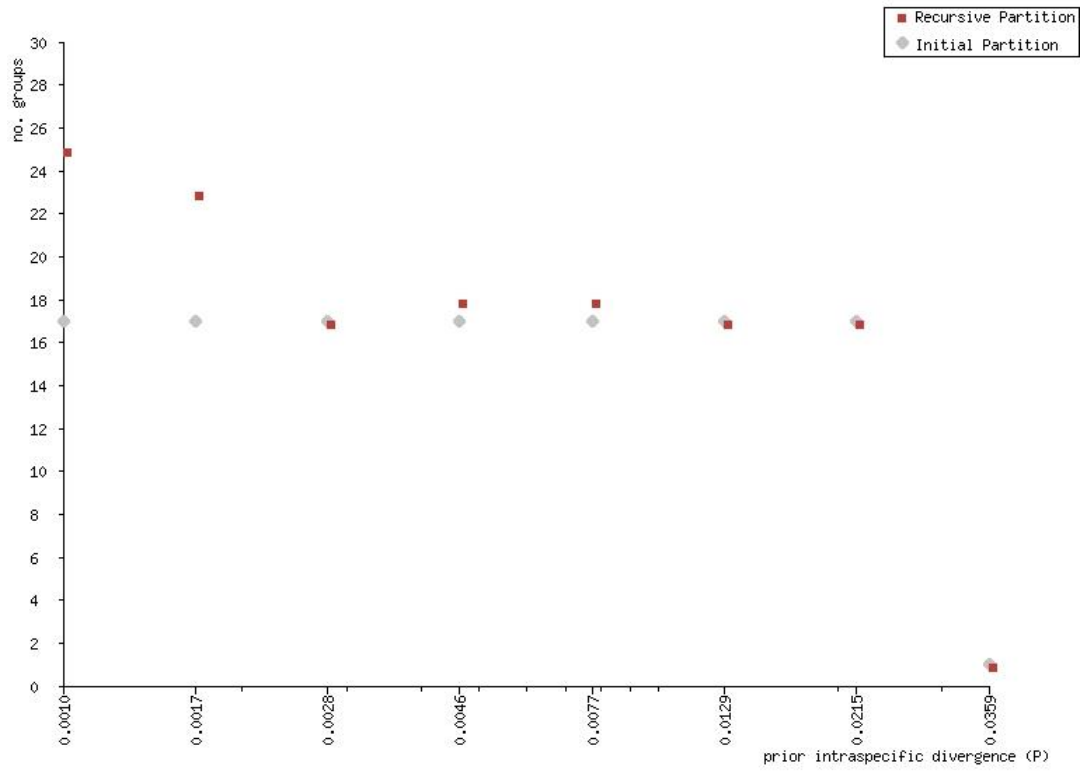
Supplementary Fig. 9. Bayesian phylogenetic tree for the 16S gene. Vertical brace on the right indicate the clades detected by the tree-based GMYC, PID, the distance-based ABGD approach, the character-based CAOS and the final assignment. The clades highlighted in pink were also detected by *rbcL*, 16S and *tufA* gene loci. For samples colored in red, 16S, *rbcL* and *tufA* sequences were also available. For samples colored in blue, two of 16S, *rbcL* and *tufA* sequences were available.



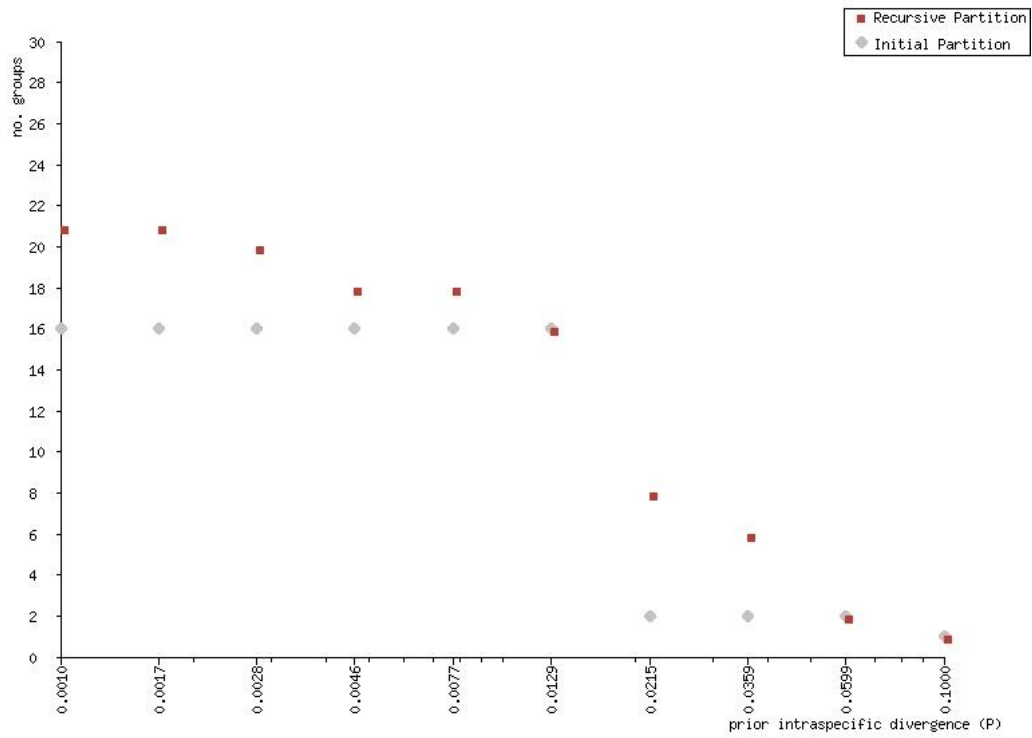
Supplementary Fig. 10.
Neighbor joining tree for
the 16S gene.

Supplementary Fig. 11.
Maximum likelihood tree for
the 16S gene.

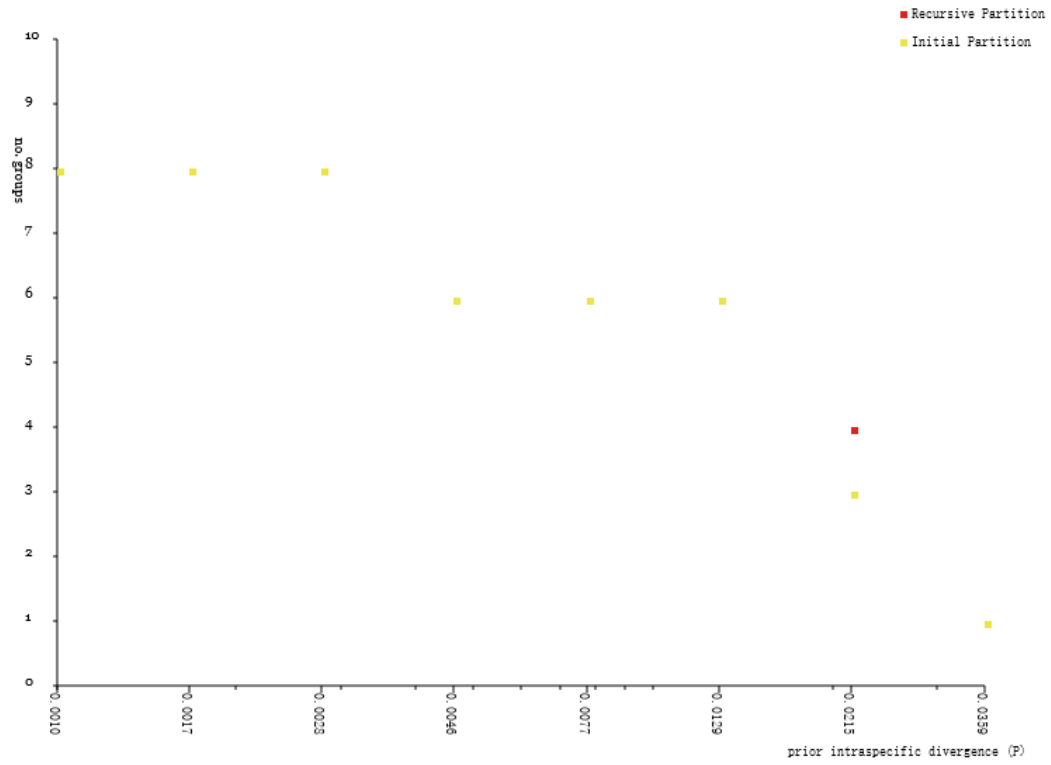




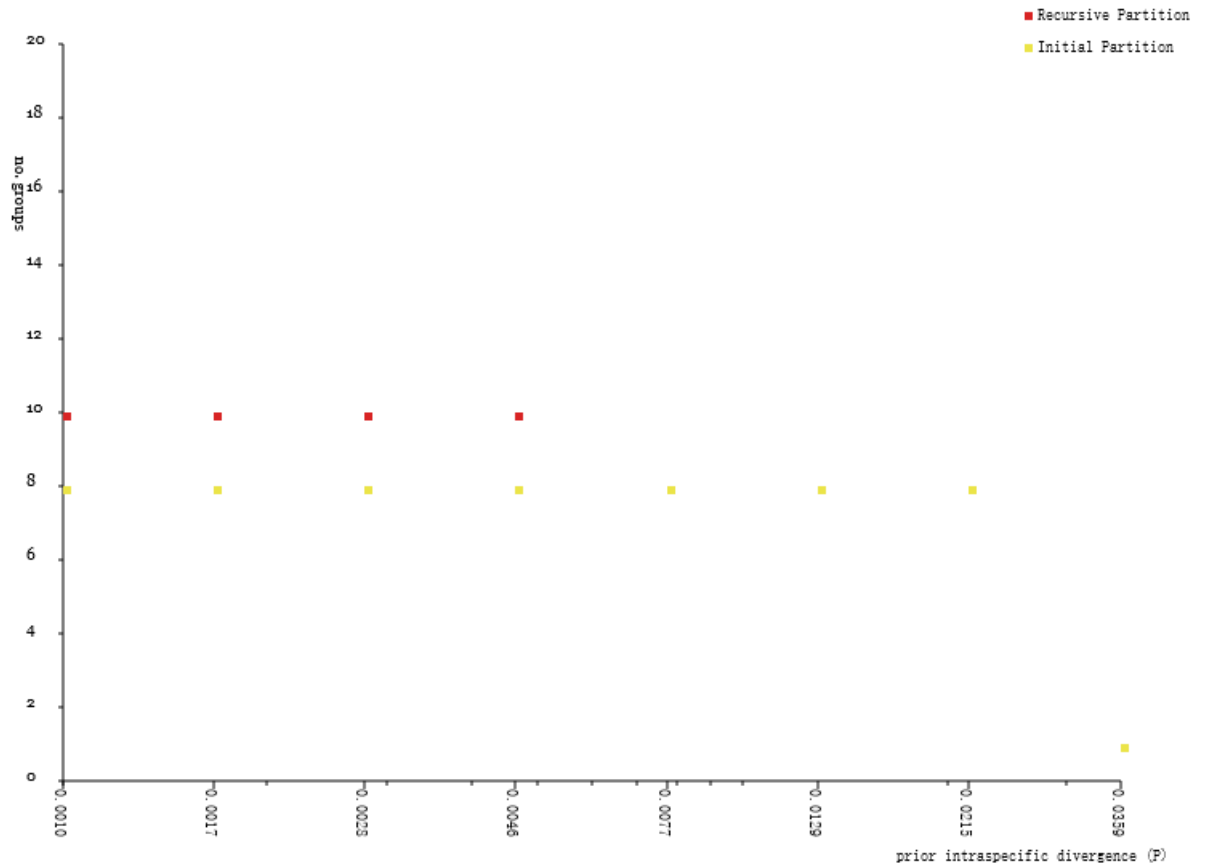
Supplementary Fig. 12. Automatic partition of tellinaceans based on *rbcL* gene. The number of groups inside the partition (initial and recursive) of each given prior intraspecific divergence value were reported.



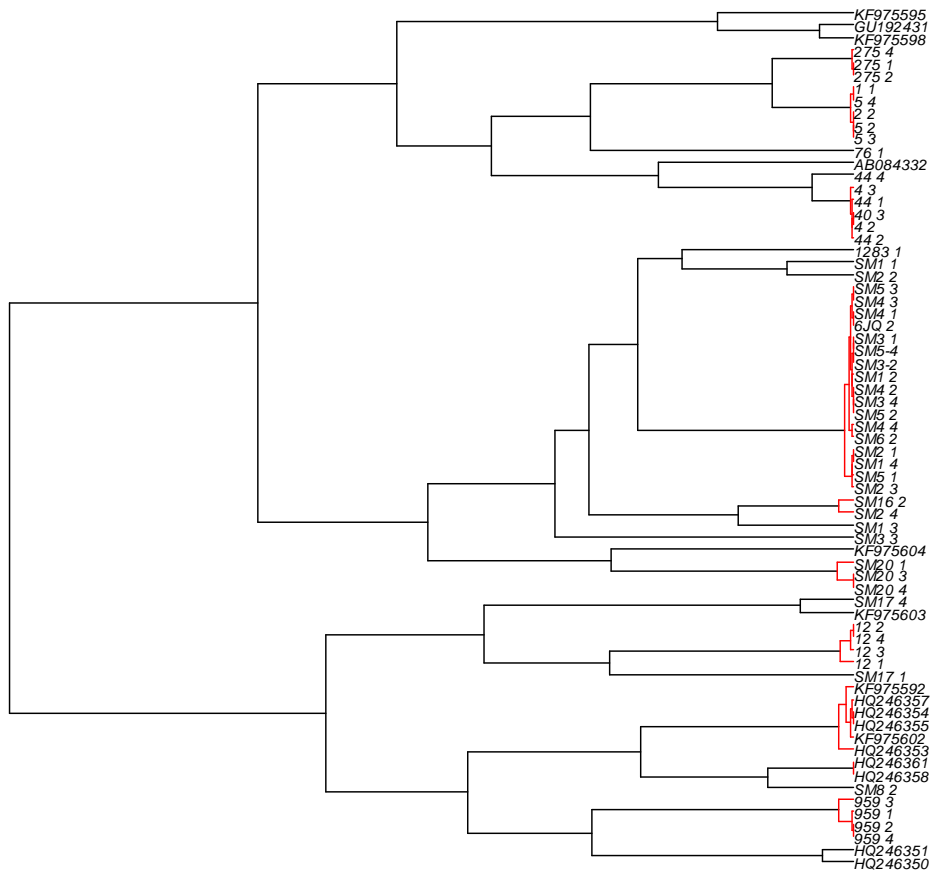
Supplementary Fig. 13. Automatic partition of tellinaceans based on ITS gene. The number of groups inside the partition (initial and recursive) of each given prior intraspecific divergence value were reported.



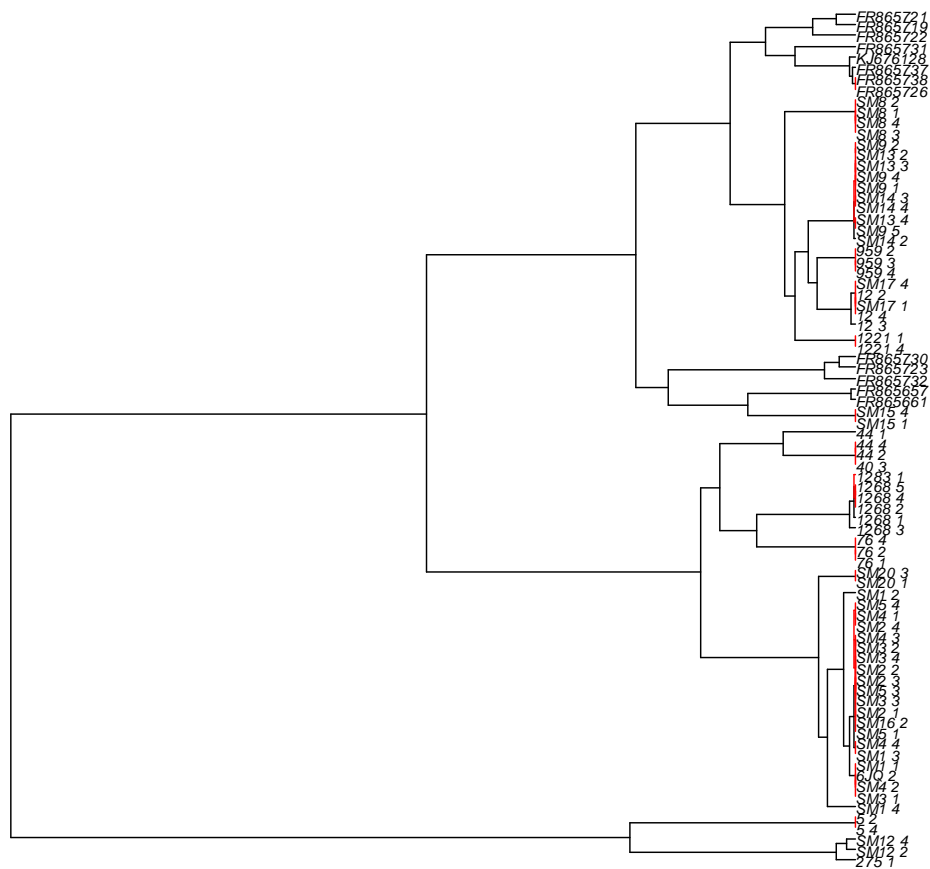
Supplementary Fig. 14. Automatic partition of tellinaceans based on 16S gene. The number of groups inside the partition (initial and recursive) of each given prior intraspecific divergence value were reported.



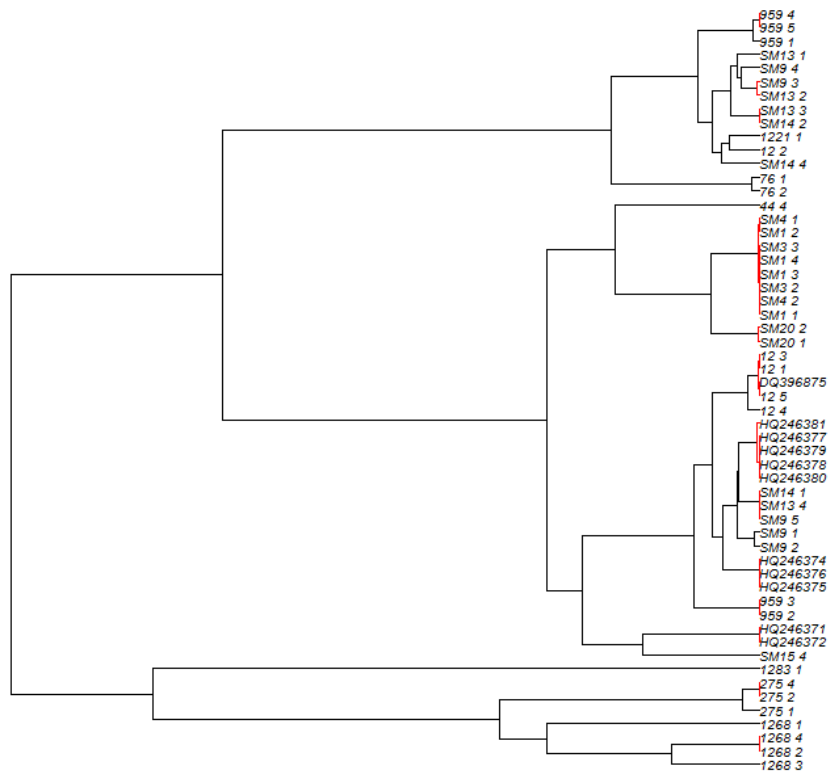
Supplementary Fig. 15. Automatic partition of tellinaceans based on *tufA* gene. The number of groups inside the partition (initial and recursive) of each given prior intraspecific divergence value were reported.



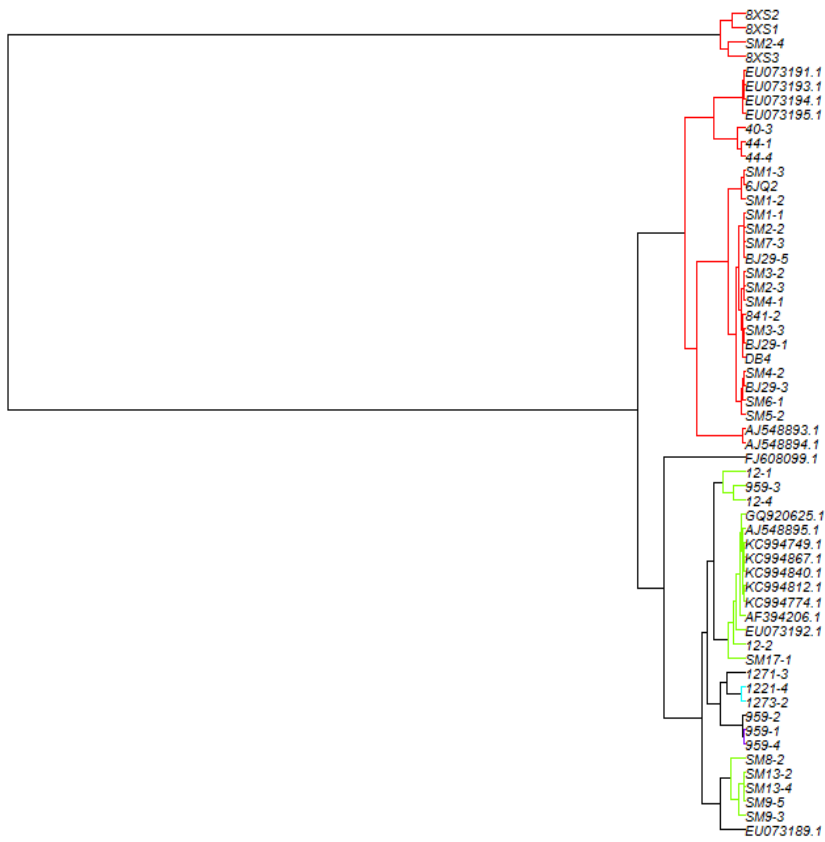
Supplementary Fig. 16. Genetic groups recovered by GMYC model for *rbcL*.



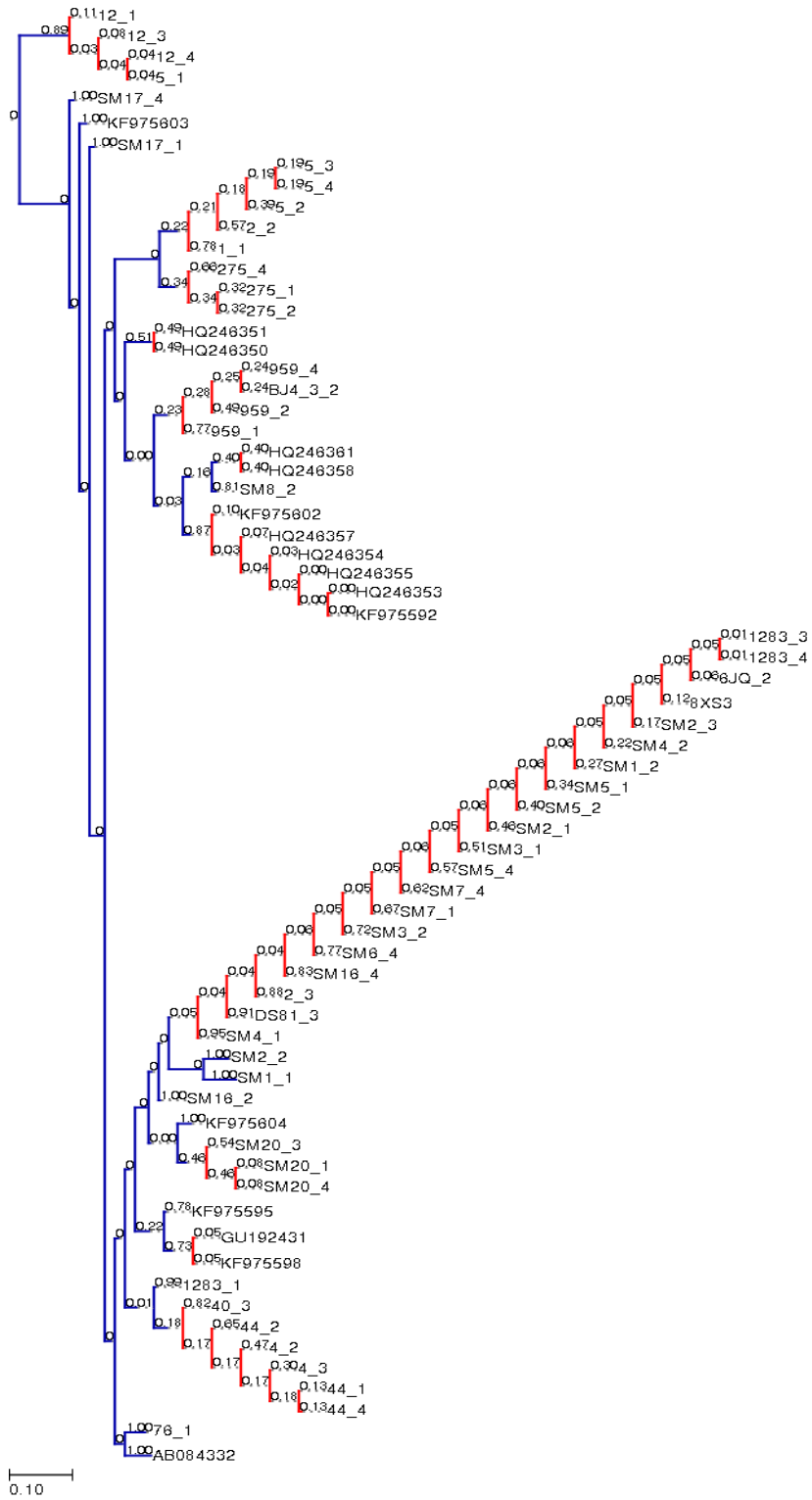
Supplementary Fig. 17. Genetic groups recovered by GMYC model for ITS.



Supplementary Fig. 18. Genetic groups recovered by GMYC model for *tufA*.



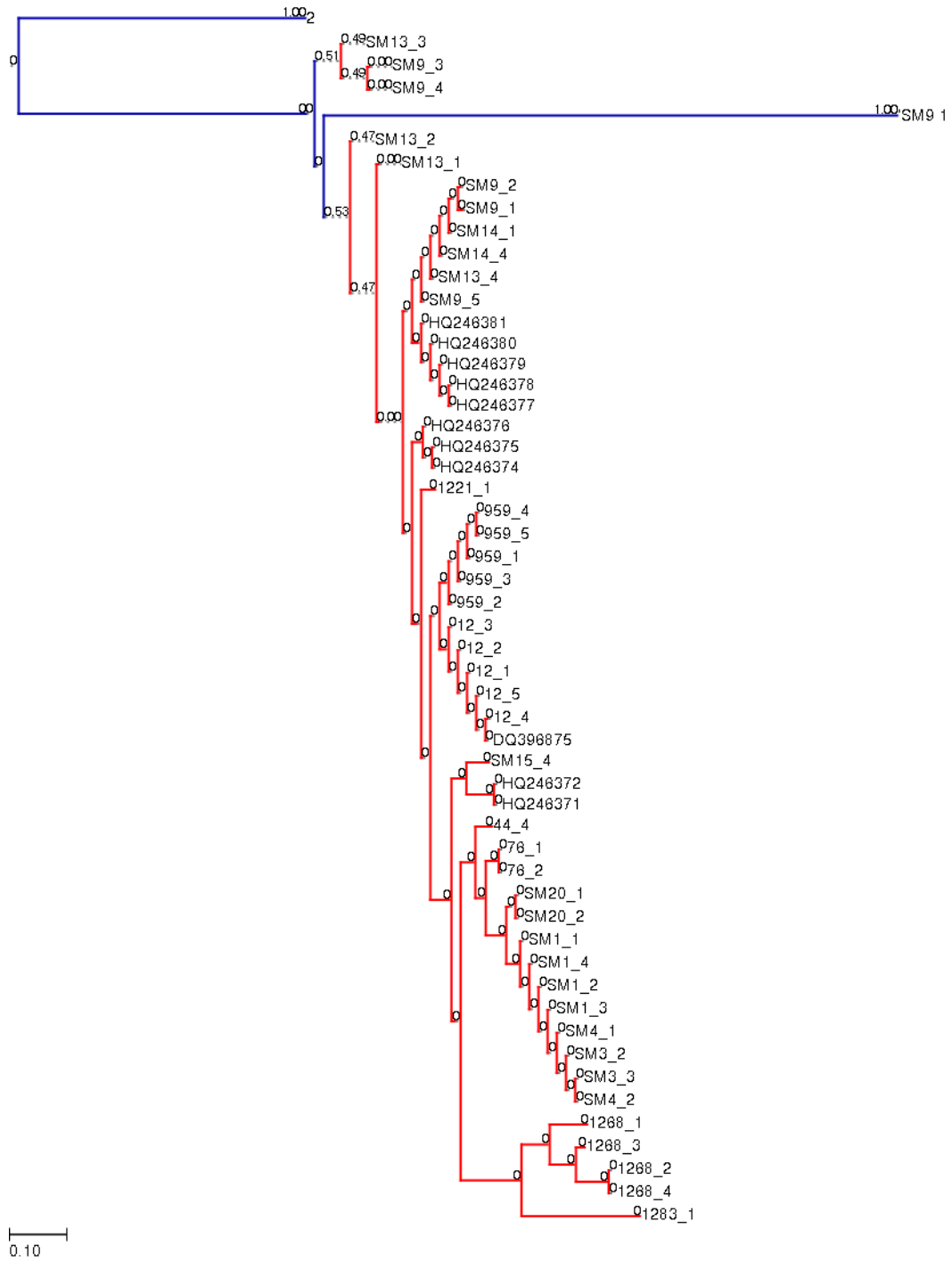
Supplementary Fig. 19. Genetic groups recovered by GMYC model for 16S.



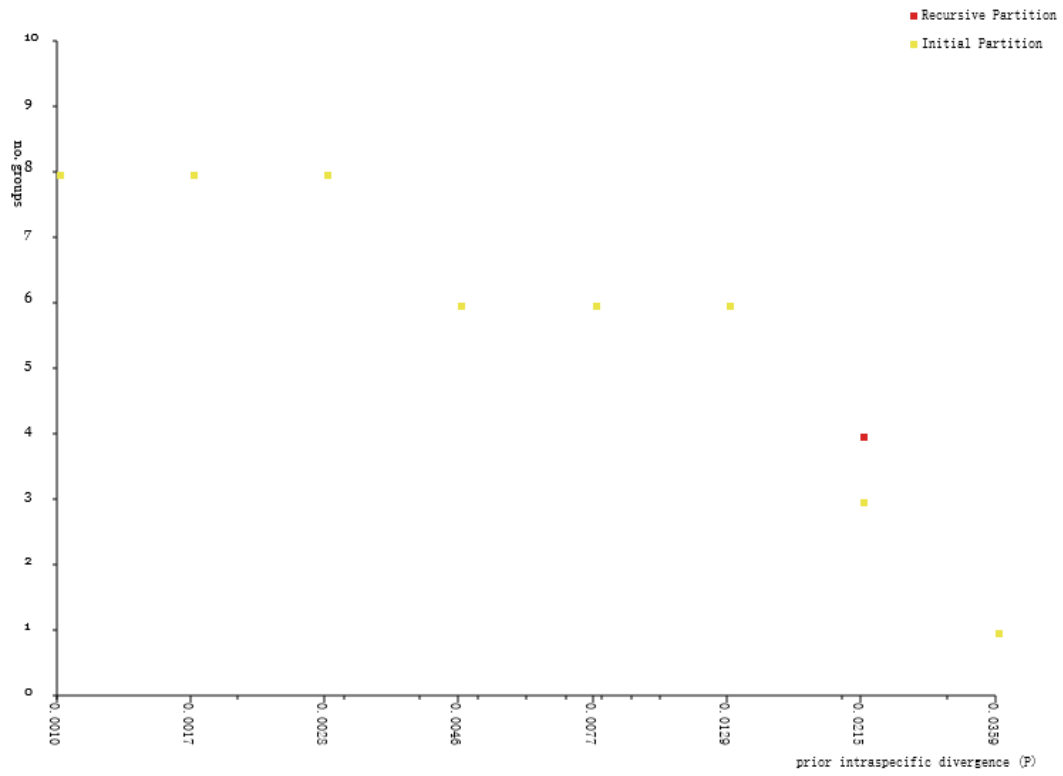
Supplementary Fig. 20. Genetic groups recovered by dPTP analysis for *rbcL*.



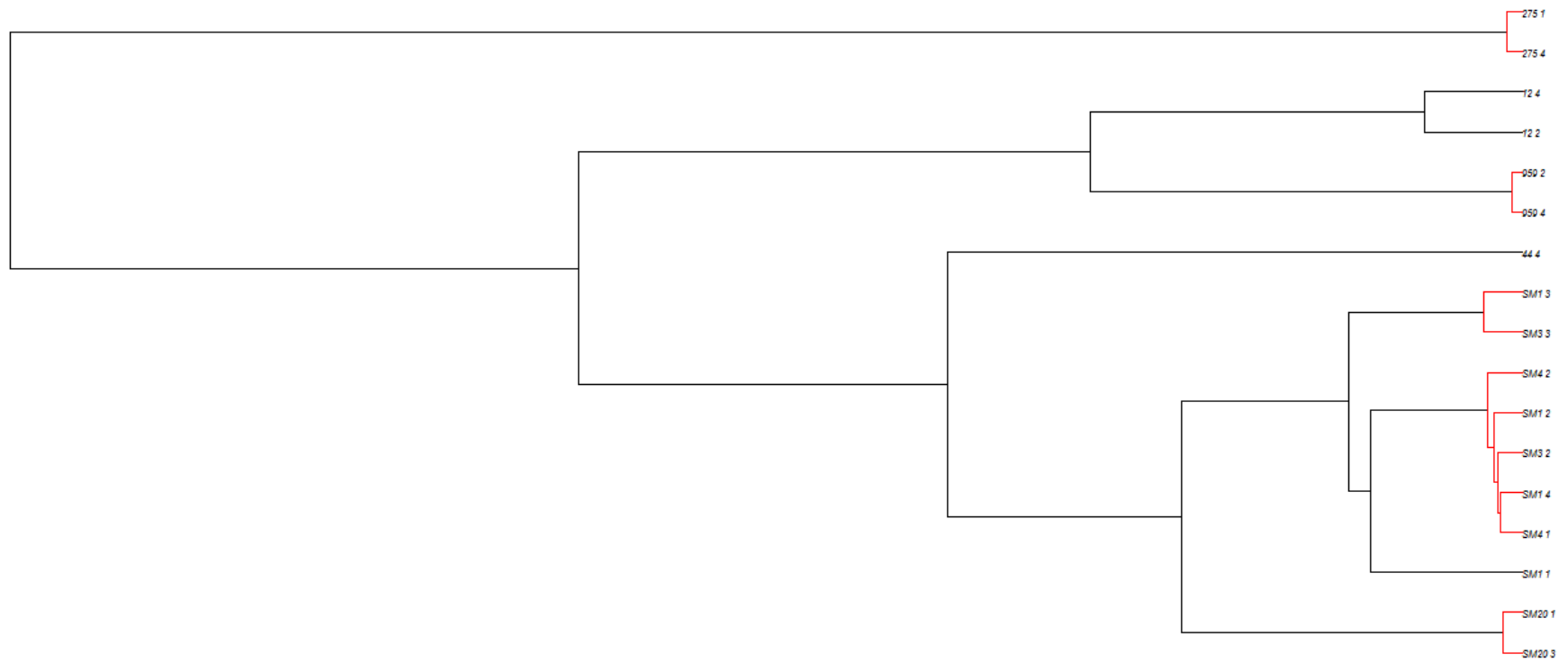
Supplementary Fig. 21. Genetic groups recovered by dPTP analysis for ITS.



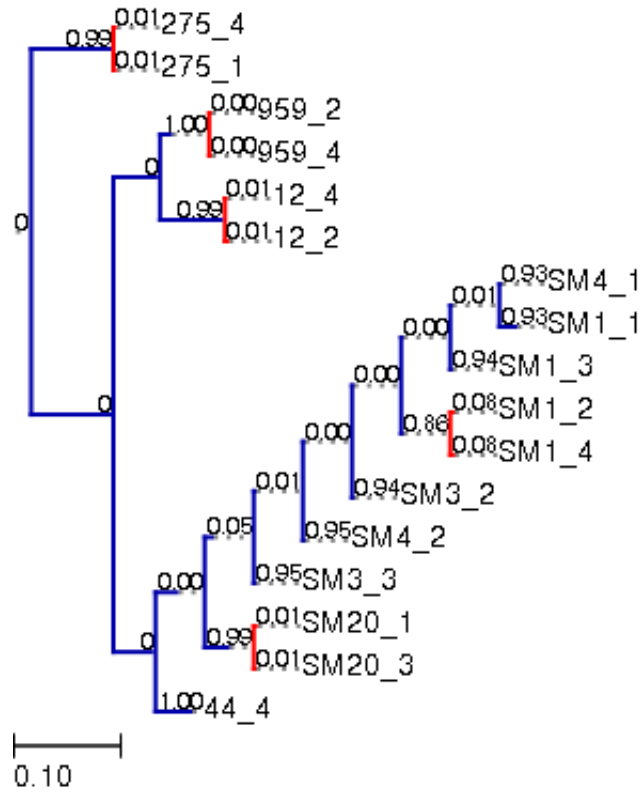
Supplementary Fig. 22. Genetic groups recovered by dPTP analysis for *tufA*.



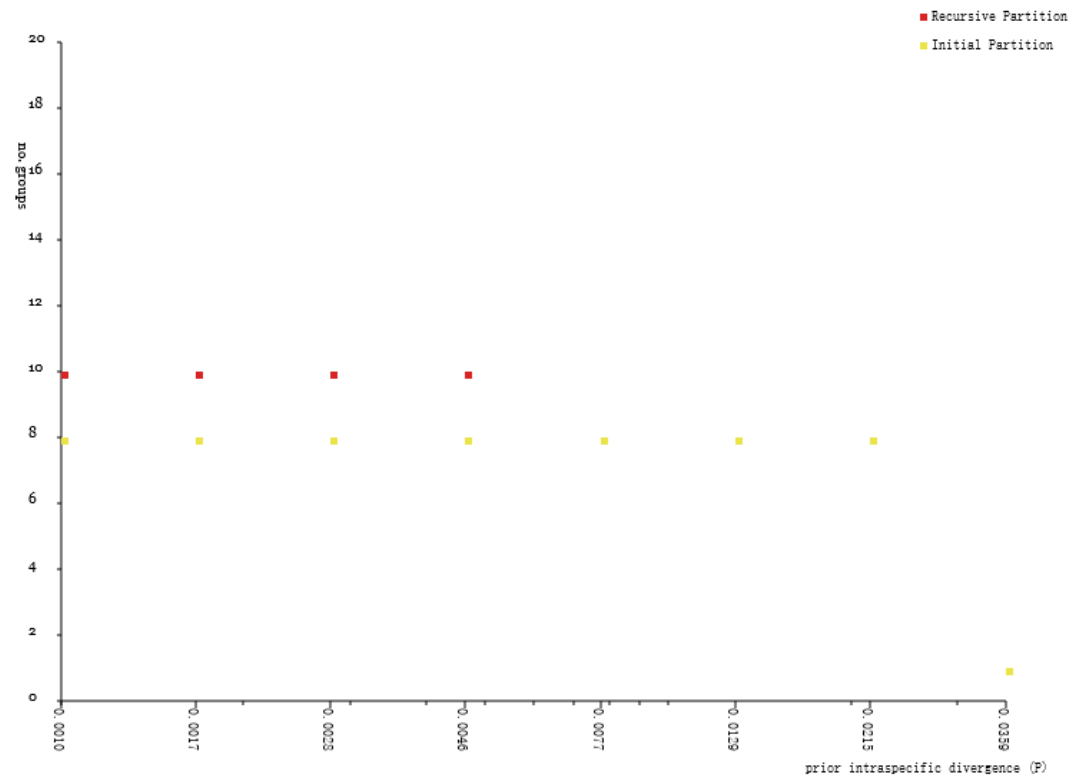
Supplementary Fig. 24. Automatic partition of tellinaceans based on *rbcL*+ITS+16S+*tufA* sequences. The number of groups inside the partition (initial and recursive) of each given prior intraspecific divergence value were reported.



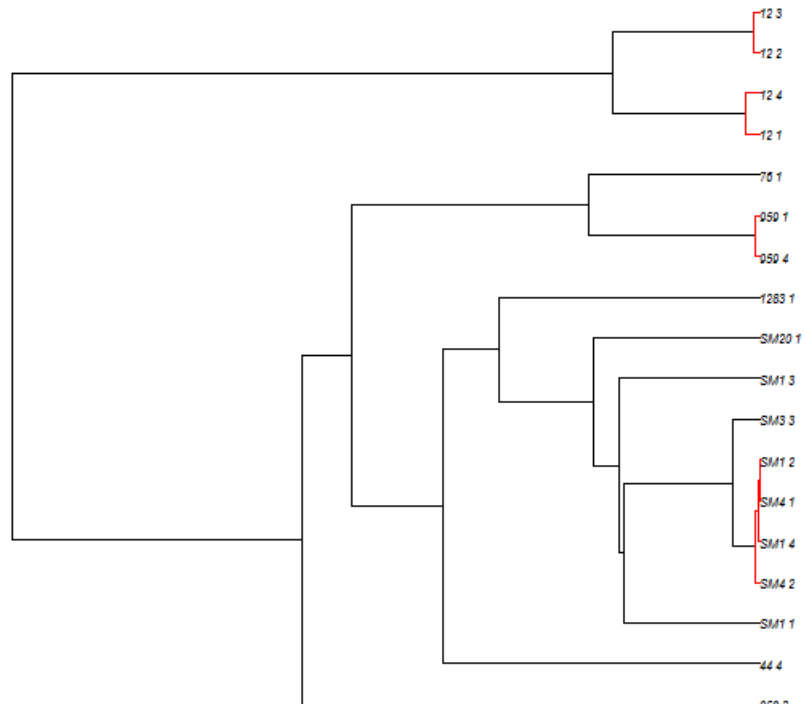
Supplementary Fig. 25. Genetic groups recovered by GMYC model for *rbcL*+ITS+16S+*tufA*.



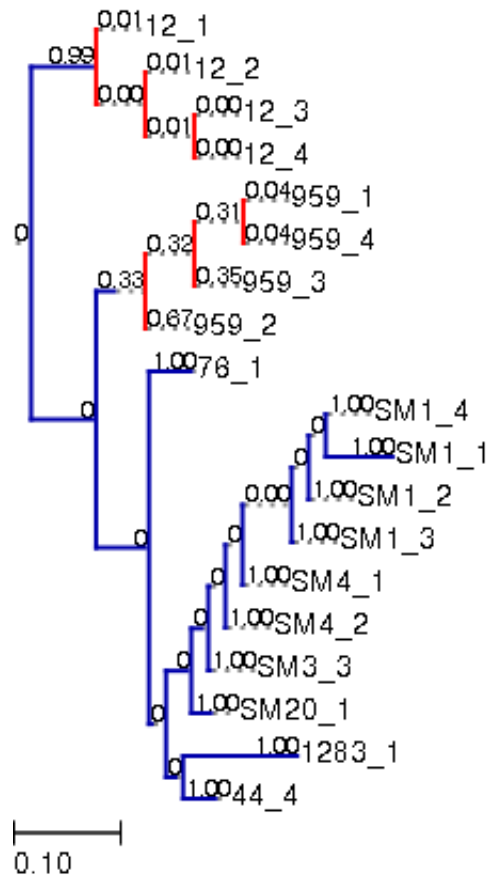
Supplementary Fig. 26. Genetic groups recovered by dPTP analysis for *rbcL*+ITS+16S+*tufA*.



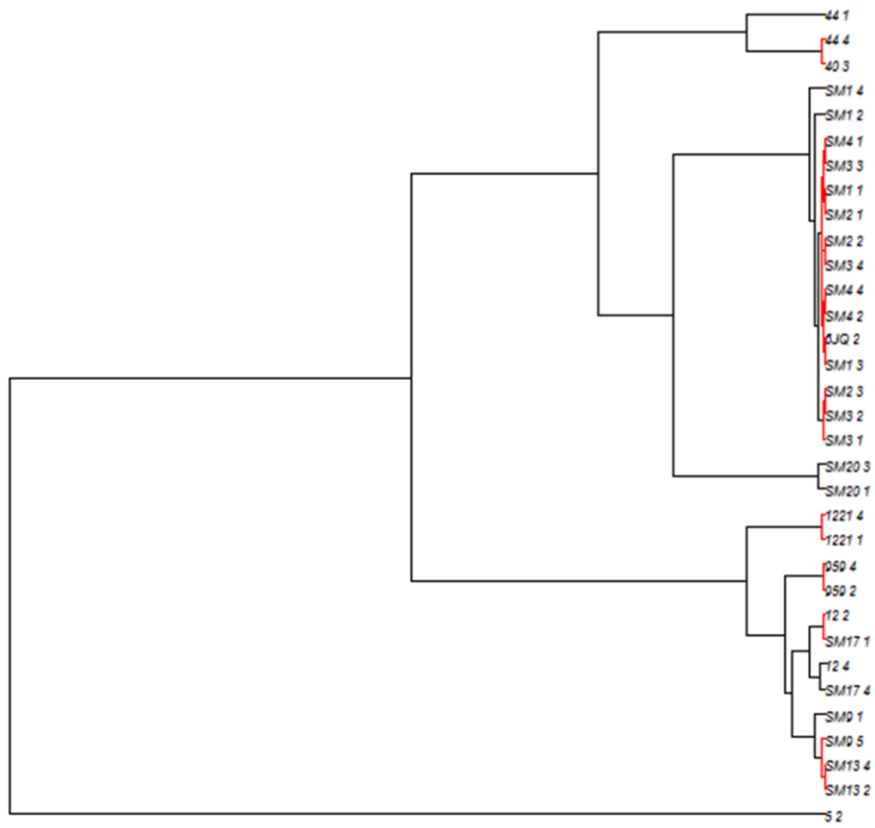
Supplementary Fig. 27. Automatic partition of tellinaceans based on *rbcL+tufA* sequences. The number of groups inside the partition (initial and recursive) of each given prior intraspecific divergence value were report.



Supplementary Fig. 28. Genetic groups recovered by GMYC model for *rbcL+tufA*.



Supplementary Fig. 29. Genetic groups recovered by dPTP analysis for *rbcL*+ *tufA*.



Supplementary Fig. 30. Genetic groups recovered by dPTP analysis for ITS+16S.

