

A morphological and molecular study of *Euglena anabaena*

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Abstract

Euglenoids represent one of the earliest divergences from the eukaryotic tree of life leading to higher plants and animals. Yet despite their evolutionary importance as early eukaryotes, euglenoids are poorly studied in terms of their own evolutionary relationships and morphological idiosyncracies. The focus of this project is to establish the phylogenetic relationships of one species of euglenoid, *Euglena anabaena*. The method for diagnosing this euglenoid species will incorporate morphological observations with the molecular sequence data. The combination of both sets of data strives to establish a congruence between the visible characteristics of the organism and its genomic information. As a result, inferences can be made as to where *Euglena anabaena* falls on the evolutionary tree and its relationship to other euglenoids.

Introduction

Euglenoids are among the most ancient of the free-living eukaryotic protists, and are one of the earliest lineages to contain mitochondria. Although possessing the unique characteristic of being neither plants nor animals, euglenoids are poorly known despite being ubiquitous in fresh water, marine, and soil habitats. An increased knowledge of euglenoids is crucial because of the ecological importance of these organisms. Euglenoids are indicators of ecological well-being; favoring waters rich in organic materials (Wolken, 1967), and can be used to indicate the health of their environment.

The organism under examination, *Euglena anabaena*, will be studied using morphological and molecular data, in hopes of establishing its phylogenetic history. Most prior attempts to establish a taxonomy of euglenoids have not used cladistic models, nor have they incorporated molecular data. Our method of establishing a taxonomy assesses a morphological and molecular congruence that may be relevant

to most protists. Such an analysis method provides a less biased method of species diagnosis for euglenoids.

Our phylogenetic analysis includes two euglenoids that are not members of the genus *Euglena*, namely, *Astasia longa* and *Khawkinea quartana*. They are included because they are believed to have lost the ability to photosynthesize. *Astasia* lacks chlorophyll and an eyespot while *Khawkinea* lacks only chlorophyll (Leedale, 1967). Analysis of their SSU rDNA (small subunit ribosomal DNA) suggests that *Astasia* and *Khawkinea* should be included in the genus *Euglena*. This conclusion is in agreement with one reached in a study by Montegut-Felkner and Triemer (1997).

Although shared morphological features among euglenoids have been used to infer species relationships, the number of characters available for species diagnosis is limited. In this work, to increase the resolution of our species diagnosis, the small subunit ribosomal DNA (SSU rDNA) molecules of *Euglena anabaena* will be isolated, sequenced, and used for comparison; the use of rDNA to resolve taxonomic differences among euglenoids has been established (Montegut-Felker and Triemer, 1997). Therefore, a phylogenetic analysis of the 18S rDNA sequence of *Euglena anabaena* will be used to infer its evolutionary position among other euglenoids. The omnipresence of rRNA among organisms makes it an excellent indicator of evolutionary relationships (Frisvad et al., 1998). Likewise, convenient techniques for primary sequence determination add to the convenience of using the rRNA gene. Before the advent of the PCR, the rRNA gene was extracted and sequenced. However, PCR-amplified rDNA regions are preferred for phylogenetic analysis and generate higher yields during nucleotide extraction.

The classification of euglenoids is controversial. Euglenoids reproduce asexually, so they cannot be distinguished according to reproductive compatibility, as can higher animals. As a result, "arbitrary distinctions ultimately are the criteria for species determination in asexually reproducing organisms such as *Euglena*" (Johnson, 1944). Past classifications of euglenoids have not accounted for the plasticity of the morphological traits. For example, Zakrys (1986) challenges the distinctions between *Euglena anabaena* var. *minor* and *Euglena anabaena* var. *minima*, stating that the observed size difference ([Figure 1a](#)) is due to the variability of the strains, and not to evolutionary distinction.

Historically, euglenoids have belonged to the kingdom Protista. However, controversy surrounds this assignment of euglenoids and a sixth kingdom, the Euglenozoa, has been proposed to house them (Cavalier-Smith, 1981).

About one third of all euglenoids are photosynthetic, whereas the other two thirds are either phagotrophic or osmotrophic. The most conspicuous feature of the

photosynthetic euglenoids is their chloroplasts; many previous taxonomic studies have been based upon this character (Pringsheim, 1956; Zakrys, 1986). Euglenoid chloroplasts assume a particular shape; for instance, the *Euglena anabaena* chloroplast assumes a shield-shaped appearance ([Figure 1b](#)). Oftentimes, as with *Euglena anabaena*, the chloroplasts contain a proteinaceous center called the pyrenoid. Pyrenoids are localized concentrations of ribulose 1-5 biphosphate carboxylase-oxygenase (rubisco), the enzyme that catalyzes the reduction of carbon dioxide to glucose. Pyrenoids may be capped with plates of paramylon ([Figure 1b](#)).

Paramylon is a beta 1,3 glucan that can be hydrolyzed to d-glucose and is found only in euglenoids (Godjics, 1953). The body of *Euglena anabaena* is spindle-shaped, as is the case for many of the *Euglena* species, and is covered by an elastic membrane complex called the pellicle. (Godjics, 1953). With the aid of this pellicle complex, many species of *Euglena* are able to engage in a unique form of wiggling movement known as metaboly. In addition, nearly all euglenoids can swim using one or more flagella for locomotion. All *Euglena* species bear a single emergent flagellum, which exits subapically at the anterior of the body ([Figure 1c](#)).

Materials and methods Culture conditions

Euglena anabaena was obtained from the culture collection at University of Texas (UTEX #373) and maintained in EG medium at 20°C under a 12 h light/12 h dark cycle.

DNA isolation

Total genomic DNA was extracted using the Chelex procedure as described by Goff and Moon (1993). This process utilized Biotechnology grade Chelex 100 resin by Bio-Rad.

Amplification of SSU rDNA fragments

The SSU rDNA coding sequence was amplified via polymerase chain reactions (PCR) using 10 microL of the DNA-containing solution from the Chelex extraction. The procedure used was that recommended by Promega biochemicals (Taq polymerase, buffer, MgCl₂ at 2.5 mM, catalogue #PR-M1861; dATP, dTTP, dCTP, dGTP, catalogue #PR-U1240). Oligonucleotide primers were used to initiate DNA synthesis in the PCR. After a 3-minute pre-incubation at 95°C, thirty amplification cycles were carried out as follows: denaturing at 94°C for 2 min, then annealing at 37°C for 2 min, then 72°C for 4 min, followed by one cycle at 72°C for 11 min. To increase amplification yields, three sets of forward/reverse primer pairs were chosen to amplify three individual regions of the SSU rDNA coding sequence ([Table 1](#)).

Purification and extraction of rDNA fragments

The amplified regions of SSU rDNA were purified by gel electrophoresis. The rDNA band was cut out of the gel, followed by a Qiaex Gel Extraction procedure as described by Qiagen.

Sequencing of rDNA: Linear amplification sequencing was performed on the purified template as defined by the manufacturer in the Stratagene Cyclist DNA sequencing kit (Cat No. 200325). The radioactive label used was alpha-[³³P] -dATP (DuPont NEN, Boston, MA). The sequencing cycles were performed as follows: 30 cycles of denaturing at 94°C for 1.5 min, annealing at 55°C for 1.5 min, and extension at 72°C for 1.5 min. Gel electrophoresis was carried out on a 6.0% and 8.0% (19:1 acrylamide: bisacrylamide) gel (National Diagnostics' Sequagel System, Atlanta, GA). In separate experiments, the purified template was also sequenced by using the ABI automated sequencer at Robert Wood Johnson Medical School per their protocol.

Alignment of rDNA

The sequence of *Euglena anabaena* was added to the aligned sequences obtained from the SSU rDNA databank at the University of Antwerp (maintained by Van de Peer, Nicolai, De Rijk, and De Wachter). The sequences were maintained on the GDE package software (Smith et al., 1994) on a Sparc 2 workstation. The primary sequence was aligned based on the secondary structure model of *Euglena gracilis* provided by University of Antwerp ([Figure 2](#)).

Phylogenetic analysis of sequence data: Using a set of 800 unambiguously aligned positions, a parsimony analysis of 18S sequence data was performed using The Phylogeny Inference Program 3.5 (Phylip 3.5) on the Sun 2 workstation and with PAUP* 4.0d63 (Swofford 1996) on a Powermac 7600/132. Parsimony analysis attempts to explain the given data using the fewest number of steps possible.

The data from *Euglena anabaena* were matched against data for nine other euglenoids; *Astasia longa*, *Euglena viridis*, *Euglena pisciformis*, *Euglena gracilis*, *Euglena* sp., *Khawkinea quartana*, *Euglena acus*, *Euglena spirogyra*, and *Eutreptiella* sp. as the outgroup ([Figure 3](#)). The data set was scored with a binary code, depending on the alignment of the primary structure of all 10 organisms. A score of "1" was given to each unambiguously aligned position. Scores of "0" were given where alignment was impossible. A majority rule analysis was performed on the data set as well, using 500 replicates, to assess the robustness of the tree.

Results and discussion Morphological analysis

The taxon *E. anabaena* has been treated differently by various authors. Mainx (1926/27) described three variants of *Euglena anabaena*: *E. anabaena* typ., *E. anabaena* var. *minor*, and *E. anabaena* var. *minima*. Pringsheim (1956) and Johnson (1944) acknowledge the three varieties of *E. anabaena*, however Zakrys (1986) asserts that *E. anabaena* var. *minor* and *E. anabaena* var. *minima* are synonymous. Pringsheim (1956) also concludes that *E. anabaena* is closely related to *E. thinophila* based on measurements taken by Skuja (1939), and in fact may be considered to be the same.

The dimensions given by Johnson (1944) were 36-45 microm by 16-22 microm. However, my observations of *E. anabaena* var. *minor* (UTEX # 373) yielded dimensions of 27.5-30 microm by 15-20 microm. One visible flagellum, located at the anterior end of the cell, is approximately one-half of the body length. There is also one prominent eyespot ([Figure 1c](#)).

The shape of the cell is fusiform with a weakly spirally striated pellicle; this pellicle allows slight metabolic movement. *Euglena anabaena* is an active swimmer, and moderately quick in relation to others within the genus; when stationary, there is moderately active metabolism.

The chloroplasts (chromatophores) are 6-10 in number and assume a "shield" shape. All of the chloroplasts contain central pyrenoids; in all observed cases, paramylon grains cap the pyrenoids ([Figure 1b](#)). However, paramylon grains were not observed freely floating in the cytoplasm.

Molecular data analysis

The size of the 18S SSU rDNA gene of *Euglena anabaena* is estimated to be about 2.3 Kb. In this study, 800 bases were aligned and used in the analysis. Of these, 138 were parsimony informative. Parsimony analysis of the data matrix yielded two trees of 484 steps. In both trees, *Euglena acus* and *E. spirogyra* form a single clade. *Khawkinea quartana*, *Euglena gracilis*, *E. species*, *E. pisciformis*, and *Astasia longa* consistently group together. The trees vary only in the positioning of *E. viridis* and *E. anabaena*. In one case, *E. anabaena* diverges prior to *E. viridis* and in the second tree the opposite was true.

A majority rule consensus tree was run with 500 bootstrap datasets ([Figure 3](#)). Two major clades exist within this group of organisms, one containing *Euglena acus* and *Euglena spirogyra*, and a second containing the remainder of the taxa. The *Euglena acus* and *Euglena spirogyra* clade is strongly supported. Strong support is

also found for the *Euglena gracilis*, *E. species*, *E. pisciformis*, and *Astasia longa* clade. The exact position of *E. viridis* and *E. anabaena* remains unresolved, but there is strong support for their inclusion into the clade containing *Khawkinea quartana*, *Euglena gracilis*, *E. species*, *E. pisciformis*, and *Astasia longa*.

The parsimony analysis tree illustrates congruence between the molecular and morphological data for these organisms. *Euglena acus* and *Euglena spirogyra* both have small disk-shaped chloroplasts that lack pyrenoids and paramylon caps. *Euglena anabaena* is consistently placed with *Astasia longa*, *E. pisciformis*, *E. gracilis*, *E. sp.*, *E. viridis* and *Khawkinea quartana*. All of these organisms also share similar chloroplast features. Their chloroplasts all contain pyrenoids and are capped with paramylon grains. With the exception of *E. viridis*, all of these chloroplasts are shield-shaped and have a single, centrally located, capped pyrenoid. In *Euglena viridis*, the chloroplast is a stellate structure with a single pyrenoid capped by numerous paramylon grains. The chloroplast forms many ribbonlike extensions that radiate out from the pyrenoid. The morphological data would suggest that the *E. anabaena* should be more closely related to the former taxa than to *E. viridis*. This interpretation is consistent with the majority rule tree but lacks strong bootstrap support.

In summary, morphological features of the chloroplast have been extensively used to diagnose *Euglena* species. The molecular data are congruent with the chloroplast data and support the use of the chloroplast as a key morphological character for distinguishing *Euglena* species.

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References

Cavalier-Smith, T. 1981. Eukaryotic Kingdoms: Seven or Nine? *Bio Systems*. 14:461481

- Ehrenberg, C. G. 1830. Neue Beobachtungen über blutartige Erscheinungen in Ägypten, Arabien, und Sibirien, nebst einer Übersicht und Kritik der früher bekannten. Pogg. Ann. Physik Chem. 94:477-514.
- Frisvad, Jens C., Bridge, Paul D., Arora, Dilip K. 1998. Chemical Fungal Taxonomy. Marcel Dekker. Inc., New York. pp. 51-76.
- Godjics, M. 1953. The Genus Euglena. The University of Wisconsin Press, Madison. pp.268.
- Goff, L. and Moon, D. 1993. PCR amplification of nuclear and plastid genes from algal herbarium specimens and algal spores. *J. Phycol.* 29:381-4
- Johnson, L.P. 1994. *Euglena of Iowa*. Trans. American Microscopical Soc. 43: 97135 Leedale, Gordon F. 1967. Euglenoid Flagellates. Prentice-Hall Biological Series. pp. 27-33.
- Lemmermann, E. 1913. Eugleninae. *Die Susswasserflora Deutschlands, Osterreichs und der Schweiz*, ed. A. Pascher, Vol. 2, 115-174. Jena : Gustav Fischer Verlag.
- Mainx, F. 1926. Einige neue Vertreter der Gattung *Euglena* Ehrenberg mit Unterstützung der Gesellschaft zur Förderung deutschen Wissenschaft, Kunst und Literatur in Böhmen. *Arch. Protistenk.* 54:150-160.
- Mainx, F. 1927. Beiträge zur Morphologie und Physiologie der Euglenen. I. Morphologische Beobachtung, Methode, und Erfolge der Reinkultur. II. Untersuchung über die Ernährungs- und Reizphysiologie. *Arch. Protistenk.* 60:305414.
- Montegut-Felkner, A.E. and Triemer, R.E. 1997. Phylogenetic relationships of selected euglenoid genera based on morphological and molecular data. *J. Phycol.* 33:512-519.
- Pringsheim, E. G. 1956. Contributions towards a monograph of the genus *Euglena*. *Nova Acta Leopoldina* 125:1-168.
- Skuja, H. 1939. Beiträge zur Algenflora Lettlands II. *Materiali Latvijas alga florai II*. *Acta Horti. Botan. Univ. Latviensis*, Riga 11/12:41-169.
- Smith, S. W., Overbeek, R., Woese, C.R., Gilbert, W. and Gillevet, P.M. 1994. The genetic data environment: an expandable GUI for multiple sequence analysis. *Comput. Appl. Biosci.*, 10:671-675

Sogin, Mitchell L.; Elwood, Hille J.; Gunderson, John H. (1986): Evolutionary diversity of eukaryotic small-subunit rRNA genes. *Proc.National Academy of Science USA*. 83:1383-1387.

Swofford, D.L. 1996. PAUP*: Phylogenetic analysis using parsimony (and other methods), version 4.0. Sinauer Associates, Sunderland, Massachusetts.

Wolken, Jerome J. 1967. Euglena. Appleton-Century-Crofts. pp.10-20.

Zakrys, B. 1986 Contribution to the monograph of Polish members of the genus *Euglena*. *Nova Hedwigia*. 42:491-540.

Table 1. PCR Primers

<u>Forward</u>	<u>Sequence 5'to 3'</u>	<u>Length</u>
1(A) ZB	ACC CTG GTT GAT CCT GCC AGT	21 467 (300)
AG	17 1055 GGT GGT GCA TGG CCG	15
<u>Reverse</u>	<u>Sequence 5'to 3'</u>	<u>Length</u>
516	ACC AGA CTT GCC CTC C	16 1913 Eug
CAG AC	20 1520 (B) ZB TGA TCC TTC TGA AGG TTC ACC TAC	24

Table 2. Interleaved data matrix used for sequence analysis

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1 11 21 31 41 50
| | | | |
E._graci AAT-CTGGT- TGA-TCCT-- -GCCAG-C-A GT-C-ATATG CT---T-TG-

Eug._sp. ..C..... ..T..

Astasia_ ..C..... ..T..

E.piscif ..C..... ..T.. ..M..

Khaw.qua aac.ctggt. tga.tcct.. .gccag.t.. ..C..

E_anabae ---.--.... ..G...T.. ..C.C..

Eacus_co ..C..... ..T.. ..C..

E._spiro ..C..... ..T.. ..C..

E.viridi aac.ctggt. tga.tcct.. .gccag.t.. ..C..

Eutrept_ ---.----- .---.----- .-----.- -.-.-. ....CT.

51 61 71 81 91 100
| | | | |
E._graci T-TCAAGGGC TAA-GCC-A- TGC-AC-GT- CTCA-G-C-G CAA-AC-G-G

Eug._sp. ....

Astasia_ ....

E.piscif ....

Khaw.qua ....T....

E_anabae A.C....A. ....a. ....T....A ....

Eacus_co ....A. ....T.... T.C....

E._spiro ....A. ....T.... T.C.... E.viridi

A.....T....T....

Eutrept_ C.....A. ....A.... c.....A.A T.C..T....

101 111 121 131 141 150
| | | | |
E._graci ---AGTGA-- -C-A-GT-GG AT-C-T-GT- GAATGG-CTC -CTTACAT-C

```

```

Eug._sp. ....
Astasia_ ....C....
E.piscif ...T.CA... ....C....
Khaw.qua .TCTCGA... .... G.....
E_anabae ...tTCA... ....A G.....
Eacus_co ...CT.CCCA ..... G.....
E._spiro ...CT.C... .T..... G.....
E.viridi ...TC..... .A....A.A. G.....
Eutrept_ ..g.T.g... .g.....SA ..... .A.....C..
151 161 171 181 191 200
| | | | |
E._graci AG-CA-GT-C A-TC-TACG- --TGA--TAG AGTG---TGC TC-----G
Eug._sp. ....
Astasia_ ....C....
E.piscif ..... .T.....
Khaw.qua .....G... ..... CT.....A
E_anabae .g..... .yTnn ....r....A ...g...C.G CT.....A
Eacus_co .....C... .....G AT.....
E._spiro .....GA.. .....C .....G A.....
E.viridi .....T.. ..C..... C.....G .T.....
Eutrept_ G...g.AC.A .....GA-. ....--C T...CAC..G .TGCTT... 201 211
221 231 241 250
| | | | |
E._graci GTCC-ACCTG CAA----- --GG----AC C--CCA-T-- -----TG-
Eug._sp. ....
Astasia_ ...A...T.. ...TGTG... ..T..GA... .....-T...
E.piscif ...A..A-.. ..G..... .T..G... .....-T...
Khaw.qua .CA..CAACC ATTGGCAG... ..T....G. T...AGa... .....C..
E_anabae ..G..C.AAC TGCCAGG... ..T..... T..-..T... .....n..

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```

Eacus_co .CA..CA-.C .CTGCCAG.. ...T....G. ...-.GC... .....
E._spiro ..G..C...C .TGCCAG... ...C....G. ....A..... .....
E.viridi .CA..CA.CT ACCAAAG... ...T....G. ....AG.... .....
Eutrept_ .GG..-...C GTG..... ...T....C. ....--.-... .....

251 261 271 281 291 300

| | | | |

E._graci --GACAT--- ----- -C-CA-C-C- A-AAACC-T- T-G-TG-G-C
Eug._sp. ....
Astasia_ ....
E.piscif ....T. ....
Khaw.qua .....AT. ....T....
E_anabae ....T.... ....T... G.....
Eacus_co ....T.... ....A.....
E._spiro ....T.... ....T.... ....A.....
E.viridi ....T.... ....A.....
Eutrept_ .T....CGG TCCGGATATT ...-..... T.C...T.G. G.....

301 311 321 331 341 350

| | | | |

E._graci TAATA-CA-C GTTCGACCC- AG-TC-A-GC ----CAT-G- CA-A-C-ACT
Eug._sp. ....
Astasia_ .....C....A..A .....G.....
E.piscif .....T..AA.. .....G.....C
Khaw.qua .....G.. .A...C.AA. G....G... .....A... TT.C.A.GT.
E_anabae .....A.T.C..A. g....T... .....GA. ...C.A.TAA
Eacus_co .....G.T.C.... G....T... ....T.C.A. AC.....G.
E._spiro .....CAA.C.AA. G....G... ....TC.A. GC...T.GG.
E.viridi .....AAT.CAA.. G....G... .....A. GC...AT.-.
Eutrept_ .....T .GAA.C---. --.....A .GC.TG.G.. --.-.-.-C

351 361 371 381 391 400

```

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E._graci C-GG-C-AGG GAT-CC--TG TC-TCCG-GA CAGTCCCTTC -ACC-G-G--
Eug._sp. ....
Astasia_ ....-TTT .CG.A....C .T.CGAA..G G.-....CA. G--TCG.CG
E.piscif ..--.-.-- ..G.A....T C.---A..G --...ATC.. .---.....
Khaw.qua ..TTT.TCAA C.AC..... G.-----G --...GCGT GTG.T-..CC
E_anabae ..CT.-.--- ---.GG..tw .G.A---... ...CTGGCA. CC.AATG.GC
Eacus_co ..TT.GT--- ---.-.-.- --,----- --,---...C. .---.-.-.
E._spiro ..TT.G.CCA .CAT--.-.- --....CT-- --.CTG.... .---.-.-.
E.viridi ...A.G..CC ATC.GGC.- --,----- --,---... ..A.....C
Eutrept_ ...T.-.TCC .GGT-.A.- -.-,----- --,---...
401 411 421 431 441 450

```

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| | | | |
E._graci T-----  -----  -----  -----  -----GGTGG
Eug._sp. ....
Astasia_.GGGGCCCCG TCCAACCGGT .....
E.piscif ...GGCCCCG T.CAATGGGT .....A...
Khaw.qua ...GGTGC.. TCTCTGTGGT GCTGGCAGTG CACACCAGCT GGGTGTT...
E._anabae CAAAGTCTCC G.....
Eacus_co -..... ..AATCTGC CTTTGACT.. .....CA.
E._spiro -..... ..A...
E.viridi AGTTG..... .. CCCTGACTCG G....TA...
Eutrept_ -..... -----
451 461 471 481 491 500

```

```

| | | | |
E._graci C-G-GA-TGT ATGC-CCAG- --CTG-ATAC GAAG-A-C-C --AGC-G-GC
Eug._sp. ....
Astasia .....A .C..... A.....

```

E.piscifA .C..... A..... -.-
 Khaw.qua ..C....TG C-..... .C....T -.-
 E_anabae TT.....TG CAC..... .C.TCC.T -.-
 Eacus_co T.....- GC..... .C.CCTT -.-
 E._spiro T.....- GC..... .C.C.TG A..... -.-
 E.viridi T.....-A .CA..... .G..T -.-
 Eutrept_ -.-.-G GCAG.TTG.. .C..C..T C..... -.-
 501 511 521 531 541 550

| | | | |

E._graci CGC----- ----AAGGC- CA-GTG-T-G TT-GGCAT-G -G-T-T-GAC
 Eug._sp.
 Astasia_ ..T..... .ATAC..... .T.G... ..T
 E.piscifCT GAAT....- .T....
 Khaw.qua ..T....CCT GTGC....A.CCGT.C. ...A....T
 E_anabae ..T....CC GG.C....A.C.T----. .C.....
 Eacus_coCT GGA...T.G.C..tT.T---.T
 E._spiroCT GGAT..T.G.T---.g..
 E.viridi T.....CT GAC...A.G.C..... C..TG--..
 Eutrept_CC GT...CA.GGC..... C-.AA-... .G.....T 551 561
 571 581 591 600

| | | | |

E._graci TCAGG-CTGG --CCCTC--- --CGT----- G-G-----CC G---C-A-GT
 Eug._sp.
 Astasia_T.....
 E.piscifT...T.....
 Khaw.quaT....TCT T.T.....A. A...G.T..C
 E_anabaeT....T.. .GAG.....A.....T .T..T.T..
 Eacus_coT...C-.....A. .T....C..C
 E._spiroT..A-...C.....A. A....CT.C

E.viridiT...C.... ..T.G..... ..A.....-. AA..A.T...

Eutrept_C.- .TTTTC-... ..T...GAT.-.. A...A..GA.

601 611 621 631 641 650

| | | | |

E._graci G-CT-G-GTG G-ATTT-CGT GCATG--CCT ----CGTGCA T-G-CC----

Eug._sp.

Astasia_ ..T....CAC... .GCG-...G.CTC C-...TCC.

E.piscif ..T....CAC.T.. .GTG...T.C---- -. ...TTC.

Khaw.quaCA ...G.G.TT. .A....GGACA.TG GCC.T.GTGT

E_anabaea...C. ...AGAGA.A .GTG...T.TAAAG C.-.--....

Eacus_coCA ...--.-.. .GTG.....C..GT C.-.--....

E._spiroCA ...--.-.. .GTG..T.TC GG...CACAG C.-.--....

E.viridiCA ...G.G.GCG .TT.T..GAGC---- -. ...--....

Eutrept_ ..G..T.C.A C.CA.-.-A A----.--------- -. ...--....

651 661 671 681 691 700

| | | | |

E._graci -----C CAC-----TT

Eug._sp. Astasia_ ..GGGGGAAC GGCCGCCC..

TG.....

E.piscifGGGGGT GGTTCATCC.. TG.....

Khaw.qua CACTGGACAC CCAGAGCC.G TG.....

E_anabae CA..TATCAT GCA..... TG.....

Eacus_co .A..... .CAGCA...T TG.....

E._spiro .A.....T TG.....

E.viridi GCATTAACAT .G.....

Eutrept_- TG.....--

701 711 721 731 741 750

| | | | |

E._graci GA--TC-G-- ---CAA---- GAGC-TT-CT G-A-CCT-A- T-CA-GCT--
 Eug._sp.
 Astasia_ ...C.....
 E.piscif
 Khaw.quaA..G.... ..A.....
 E_anabaeA..TG... ..A..... ..C..
 Eacus_coA..C..... ..C..
 E._spiroA..A..... ..C..
 E.viridiTC.... ..A.....T.....
 Eutrept_ --....CA..GTGT.T.C....C
 751 761 771 781 791 800

| | | | |
 E._graci T--G-ACTGT GGT-G-T-AT C-G-G-ACCA CAGTGGC-CT TG-A-C-GGG
 Eug._sp.
 Astasia_ C..... T.....
 E.piscif T.....T.
 Khaw.qua .G.....CG T.....
 E_anabae .T.....C..G T.....A....A. .A.....
 Eacus_co .T....TG.. T.....CA....AG
 E._spiro .G....TG..G T.....CA....GG
 E.viridi -GT...T...G T.....A....TC .T.....A.
 Eutrept_ .G...TTG..G T.....CA....TA .C.....
 801 811 821 831 841 850

| | | | |
 E._graci -TAA-CGGAG AAT-CAGG-G TTC-GAT-TC CG--GAG-A- GG-G-AGC-C
 Eug._sp.
 Astasia_A..T
 E.piscif
 861 871 881 891 901 911

```

Khaw.qua .....

E_anabae .....T.... ..T.....

Eacus_co ..... ..T.....

E._spiro ..G..... ..c...C...

E.viridi .....

Eutrept_ .....GA ..... ..T.....

851 861 871 881 891 900

| | | | |

E._graci TGAGAGACG- GCT-A-CC-A -CTAC-CAAG -GTGG-GCA- GCAGGC-ACG

Eug._sp. ....

Astasia_ .C.....

E.piscif .....C...

Khaw.qua .....A.... .....G..

E_anabae .....G..

Eacus_co .....A.... ..T..T... ..A.....

E._spiro ..... ..T..T... ..A.....

E.viridi .....G..

Eutrept_ .A...A.... ..T.G..... ..C.A.....G..

901 911 921 931 941 950

| | | | |

E._graci CAAA-T-T-G CC-CCA-TGC -----A--- AAGACAGTC- ----TGTG--

Eug._sp. ....

Astasia_ .....

E.piscif .....g .....

Khaw.qua G..... ..A...C. ....A ...C.TA..G .....G...

E_anabae .....A...R. C..... -.C..CA.G. ....G....

Eacus_co .....A..... ..T... T.C...C..C T...G....

E._spiro .....A..... C..... -. ....T..T G..TG....

E.viridi .....A..... --....CC.C .....

```

```

Eutrept_ ..... T...AC.... C.G..... --C..T.GTC ...TG.C...

951 961 971 981 991 1000

| | | | |

E._graci A-GGCA-GC- G-AC-G--AA CAGTAG--CA A---CCCCGT CGGC---CTT

Eug._sp. ....

Astasia_ ....T..T.. ....AA.

E.piscif ....C..... ..T...g. ....AA.

Khaw.qua ....T.C..... ..T..T.C Tgg....-..

E_anabae ..... A.....GT ...C..... ..TT.T.C .A.....-AC

Eacus_co ....C.C..... ..TT...C TT.T....C.

E._spiro ....T.C..... ..T....C .T.T....C.

E.viridi .....T .C.C..... ..t..T.C ..A....--.

Eutrept_ ...A..... ..G .CA..... G.....T.C TCA.....C. 1001
1011 1021 1031 1041 1050

| | | | |

E._graci ACGT----- --GCCGATGG GG--C-TTG- -GAATGG-AC ---GCTA--T

Eug._sp. ....

Astasia_ CAA..... ..A..... ..T .....

E.piscif CAA..... ..A..... ..T .....

Khaw.qua CAACAT.... ..AG... .A..... ..T ....A....

E_anabae CT..T.... ..T.G... AA..... ..aT .....

Eacus_co CT..G..... ..A..AG... AA..... ..T ....A...t

E._spiro C-..G..... ..A..AG... .A..... ..T.....T ....A.AA.

E.viridi CTC..... ..T..G... .A..... ..T ....A....

Eutrept_ GT..G..... ..TG.G... .A.CT..... .C....A..T .....

1051 1061 1071 1081 1091 1100

| | | | |

```

E._graci CCAAAGA--- -CAGCC-GT- GAGTATCAAC --CGGAG--G GC----AAGT

Eug._sp.

Astasia_T..A.. ..T......C.....

E.piscifTT.A..c... ..T......C.....

Khaw.quaC.... ..TG.A..T......aag.

E_anabaeT... ..C..T.A.. ----- ..-----.. --.....----

Eacus_co c....Ca... ..TG.A..T.....

E._spiroA.... ..TG.A..T.....

E.viridiC.... ..TG.A..TK.....

Eutrept_CG... ..A.A..T.....

1101 1111 1121 1131 1141 1150

| | | | |

E._graci -CTGG-TG-- --CCAGCA-- -GCT-GCGGT AATT-CCAG- CTC CG--AG-

Eug._sp.Yg.. a.....g. c.....

Astasia_A.....

E.piscif .c..... ..C..... ..C.A.....

Khaw.qua ...g...S..C..a.g.A.....

E_anabae .----,--.. ..-----.. .---,----- ----,-----, -----,--.

Eacus_coA.....

E._spiroA.....

E.viridiA.....

Eutrept_A.....

1151 1161 1171 1181 1191 1200

| | | | |

E._graci GGCGTATACT -AACATTG-C T-G-CTG-TT AAAAC-A-C- TT-GTA-GTC

Eug._sp.

Astasia_

E.piscif ..C.....

```

Khaw.qua A..A..... .G.....
E_anabae ----- .-----.-.-.---.--- -----.-.-. ---.---.---
Eacus_co C.....
E._spiro T.....
E.viridi ..... ..T..... C.....
Eutrept_ A.....A ...TG..... G...A.G... .C.....
1201 1211 1221 1231 1241 1250
| | | | |
E._graci -TGCCT---- --AC--GGGC -T-GCAGG-T -CTGCTGGG- T-GG-CC-GG
Eug._sp. ....
Astasia_ .....-.... ...C..... C.....A.
E.piscif .....-.... ...C..... A.
Khaw.qua ..g.-.... ...T...T .....C.A.. .....g
E_anabae .-----.... ..-.-.----- .-.-.-----.- .-.-.---.-
Eacus_co ...AT-.... ..G.T...T .....A.CT
E._spiro ...AT-.... ..G.T...T .....C.... -.-...A.CT
E.viridi .....-.... ...T...T .....
Eutrept_ .AA..CCTGG GC.TG..... ..G ..GT.C..C. C..C.T.C.C
1251 1261 1271 1281 1291 1300
| | | | |
E._graci TTTGTTGT-T -TC-TCTGGC CAGGG-AA-G G-A--C-CTC G-GTTC-GA-
Eug._sp. .... Astasia_
C.C.C..-.- .....-....T .G....CC.C C.CTG....T CG.G-..TC.
E.piscif CCG-----.- .-.-.-....- -----.-.-.-.-.T.... ...-G..g.
Khaw.qua CGG..G.C.G ..G..tCgcT .CC-A.CT.C tCTCAAC.A. T.TC.G.TG.
E_anabae -----.- .-.-.----- -----.-.-.-.-.-.---.---.
Eacus_co -GG..G.GCG TGGCG..C.T -T.CCTGTTC .C.GGTGG.- -.-.---.-.
E._spiro G.G.GGCGG. CC.T.G.TC. TCT..GTGT- -.CCTG...T ..CAGG.CCT
E.viridi C.G.A....A .GT...---- A.---.CC.A C.GT.-.-.--- -.-.---.-.

```

Eutrept_ .GG.C....C TC..CGACCG TCA.C.CG.. .TGGCTC.CG .T.GAT.--.

1301 1311 1321 1331 1341 1350

| | | | |

E._graci CCCTG-T-GT TGGG-C-T-G CAA-CGGC-T GGACT-----C

Eug._sp.

Astasia_ GT-G..G.TC AC.C..TCG. .GG..A-... ..

E.piscifG.TC .TC..GAtCA TGG.GTT... .CCT.GGCAG TGCTGGATT.

Khaw.qua TG-.T.-..C G.AA.GAGT. TGG.T.TG.G C.CG.CTCTC TGCTGGGTCG

E_anabae -----.-.- -.-.-.-.- -.-.-.-.- -.-.-.-.-

Eacus_co -----.-.- G..T..T-GC GTCTGCT.GG T.GTA.....

E._spiro -..C.G-G.G G.CC.-...- GGG.-TT-.- ---..GCCTC CCGCTGGT..

E.viridi -----.-.- -.-.-.-.-C ..T.-.-... ..G.....

Eutrept_ GTGCT.G.C. G.AT.-.-.- -.-.-.-.- -.-.-.-.-T. 1351
1361 1371 1381 1391 1400

| | | | |

E._graci AA-CC-CC-- -CAGTGG-TA CGTC-CCTGC GCCC---ACC TCT-CAGTC-

Eug._sp.

Astasia_C..... ..T.C....

E.piscifC..... T.....A. A..T.TTCT.

Khaw.qua ...A.C.T.. ..G..... T.....--- GA.TG.CCAG E_anabae

--.-.-.-.-.- -.-.-.-.-.- -.-.-.-.-.- -.-.-.-.-.-

Eacus_co T..-T..... A.....-G ...T.CA.-.

E._spiro ...AAC.... .TG..... T..-T..... A.....GTG C.AA.----.

E.viridiG..... T.....A.....GA- -G.T-.TCT.

Eutrept_ ...A.C.... ..T.CA.-. T.GTC..C.TGA- -.TG----.

1401 1411 1421 1431 1441 1450

| | | | |

E._graci ---GATGGTG -AGATCT--G CTCC----TG CCAAA----A GT-C-TG-CT

Eug._sp.C.....

Astasia_C....ATC...C.....
 E.piscifA.....C.....
 Khaw.qua T....Cg...A..... ...T.....C....C...
 E_anabae ...----- .-----.- ----,....- ----,....- --,-.--,--
 Eacus_coC..A.A... .C..... .TCC..... .C.-.--...
 E._spiroG.C...TCC..... .C....C...
 E.viridi-...C.....CC...
 Eutrept_ ...C....GT .TCGGACT..G. .A.G..... .C....C...

1451 1461 1471 1481 1491 1500

| | | | |

E._graci TCACT-GCAG GCC-AAAGC- GG-TT-TA-T GC-CTCC-CG -C-ACT-GGC
 Eug._sp. ...T.....

Astasia_AA...C..A....
 E.piscif ...T.....C.....A
 Khaw.qua ...G...G..G...G.AT..AA ...G.C....
 E_anabae -----,---- ---,-----, --,--,-,-- --,-----,-- .-,---,---
 Eacus_co ...G...GT. .MM.gct...GC.A TA..C.-.AA ...Gc..T.T
 E._spiro ...G...GG.GCT...GTGC.A TA..C.-.AA ...G...T.T
 E.viridiGG.C..... A..CC.AT.G C....GA.GA .G.CAG..C.

Eutrept_ ...G...GT. ..T.G.T... ...-.C...T.TATG.... 1501
 1511 1521 1531 1541 1550

| | | | |

E._graci AACG-GACAC CAA-CAGGGG ACCCAGC-CT CGA-GCTGGG T-AGTCTA-C
 Eug._sp.T.....
 Astasia_ C..... -G.....C ..C..... C...C..C.A
 E.piscif T.....G. -...-... ..TG..... GTG.C.A..C -...ATAC..
 Khaw.qua GT.CT.TTGT G.-.CATCC .TGGT.G.GA .AG..GCCCT C.T..GGT..
 E_anabae ----,----- ---,----- -----,-- ----,----- -,------,-
 Eacus_co GTGTT..... .C-.-Tgg aggccttgc cgtgcgtngc ctccaagtcc

```

E._spiro GTGTT...G. .C-.-..... c.A.G...-t GC-...C.T. G.-...-.-
E.viridi .T--.----- ---.----- TG..CTG.AC TA..C..CA. G.TCC.--.-
Eutrept_ GCG.ATCACT TC-..GATCC -GA.CT..GG A.C.CG.CCA ..G.ATG-..
1551 1561 1571 1581 1591 1600
| | | | |
E._graci CTCT----- ----- ---G-GTCCA CCACC-GGAG C-C-CACCGT
Eug._sp. ....
Astasia_ AC.CCC.... ....
E.piscif ..--..... ....G. ....A.
Khaw.qua ..GCAGATTC AACACACCCC ACC..... ....A...
E_anabae ----..... ....-,- ----,- ----,-,-
Eacus_co acctatcctg gggtc cmcm tccctgtc-- ----,- ----,-,-E._spiro -
--..... .CCC CA.-..... T.....
E.viridi ----..... ....-,-NNAG .....
Eutrept_ .ANCT.... ....-,- ----,- ----,-,-
1601 1611 1621 1631 1641 1650
| | | | |
E._graci C--TTCGA-- --CAC-C-CT GG---AAAAC -TCAG-T-GT G-C-T-CA-A
Eug._sp. ....
Astasia_ .A.G.-.... ....
E.piscif .aG.-.... ....
Khaw.qua .AA.-.... ....G. ....
E_anabae -.- ----. .-,-. -.- ----. -,-. -,-. -,-
Eacus_co -.- ----. .-,-. -.- ----. -,-. -,-. -,-
E._spiro ..TC.-.... ....G. .-..... ....C..C..
E.viridi .G...-.... ..G.T.....
Eutrept_ -.- ----. .-,-.G. .A....C... ....C..C ..T.C....
1651 1661 1671 1681 1691 1700
| | | | |

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```

E._graci AG----CA-T -CC-CCGCGA C--GG-C--- -TG-AATGTC CATCCA-TGG

Eug._sp. ....

Astasia_ .....T.....C. ....

E.piscif .....T.TT. ....

Khaw.qua .....G.A ....TGC... T..g.....C. TG..c....

E_anabae --....--.- --,----- -,--,-.... ,--,----- -----,--Eacus_co

-....--.- .cc.-actca t..gg.c... .tg.gatgcc cgt.....

E._spiro .....AG.- ....TwTGC- -.....G...C. .G.....

E.viridi G.....G.. ....TGTTAC T.....A

Eutrept_ .....A .A.GTGA.TT -...A.T... ....C...AT TCAG.G.C..

```

1701 1711 1721 1731 1741 1750

| | | | |

```

E._graci --AATG-TCA AGGCA--T-C G-ACCAAG-T GTGGC----A -TTGG-A--G

Eug._sp. ....

Astasia_ ....C.....G.....

E.piscif .....G ...T.....G.....

Khaw.qua .....GA.....G.... .g.CAaaT .C.....

E_anabae ..----,--- ,---,,-.- -,-----,- -----,---,---

Eacus_co .....G CA.T.....TGA.. .....- TG.....

E._spiro .....c. CA.....CC.. .CTTGGCT.T .....

E.viridi .....C...C .A....A.. ....G..C. ....-.....C.....

Eutrept_ ..G..R.G.. .A.G.....TA.. ....AC....C-A..C.T.

```

1751 1761 1771 1781 1791 1800

| | | | |

```

E._graci TTG-----T G-C-TG--CC TTGG-GG-CC CACTCTGGA- CAACCTGG--

Eug._sp. ....

Astasia_ .....G.....-.....G. ....

E.piscif .....G.....G. ....

```

```

Khaw.qua .....GG.. ....A.... ....A..G. ...TG.....

E_anabae ---.....- -.---.--- ---.---.--- -----, -----,

Eacus_co .....G... ....T.CT. AT.CAA..G. G...A.....

E._spiro .....G... ....T.... ..GGT...G. G...A.....

E.viridi .....A....G... ....AA... .T.....G. ....AC.T..

Eutrept_ ..C..... ....AGT. .C--.A.-- -GG..C.--. ...AGG.C..

1801 1811 1821 1831 1841 1850

| | | | |

E._graci TGGTGTGT-T C-CT----GC AGG-ATCAAC ----- ----AGG-AT

Eug._sp. ....

Astasia_ ...GTGT-.. ...C..... ....TC.... .GTT..... ..TGG.T.CC

E.piscif ...GTGT-.. ..TC....-- ---.----- .....T..GG

Khaw.qua ..CATG.-.. ....TCTT CTC.TC.TT. ....TCCTT.GG

E_anabae -----.- -.---.--- ---.----- .....---.---

Eacus_co ..C...CCCC GG.CAC.... ....C..TT. CTCGCGGGTT CGTGCT-.-

E._spiro CAC...--C ...C....AG T...cA..CG GGGGMMGCCC TCG.T...TG

E.viridi C.CATGT-.. ...A....-- ---.----- .....---.---
Eutrept_ AACGCA.-.. G.....G T.C.CC.C.G ATGGC..... ...CG.-AG-

1851 1861 1871 1881 1891 1900

| | | | |

E._graci -CG-TTGC-C CTGCCTG--- ----- G-CCT-TCGG G-TCTCGT-C

Eug._sp. ....

Astasia_ .GT.GGAA.. .CCGTC.TCG CCGGCTCCGG C..G..G... C.GGCGTG.G

E.piscif .AC.CACT.T GG.----... .....C T.TGG.C.TC A.CT...G.T

Khaw.qua .G-...CT.T T...TCCTCT CTGTGTCTCT C..GC.G..C T.G..GTG.A

E_anabae .---.---.- ---.---.--- .....-.-.---.---.---

Eacus_co .---.---.- ---.---.--- .....-.-.---.---.---

E._spiro ..T.---... .CCG..... .....G..GCT.. -.-.---.---.T

E.viridi .AC.CATG.A ...G--... .CGCTAG..A ..-C..A.. T.GGC.TC.A

```

Eutrept_ G.-TA.C-CG ..T.GGTGTG GTG.....GC -CTT-CA.C. -GG.AGC-TG

1901 1911 1921 1931 1941 1950

| | | | |

E._graci AGG-CTT-CG -TCC--CCT- GTC-CCTGCA G-CTT-GCAC -CC-A-

TCGEug._sp.

Astasia_ G.....G... .GTT.C..C.TG E.piscif

G.A..CA.T.A. ..A.....TG A.....

Khaw.qua CCT.G.C.AC .AG.ACG-.. C-G.TTGTGG ..A.C.A..G .GT.GAGT..

E_anabae ---.---.--- .---.---. ---.---. ---.---. ---.---. ---.---.

Eacus_co ---.---.--- .---.---. --.....TG TGG.CT.G.G GG.AGTAT-.

E._spiro G...---.--- .---.---. ---.AG..G T..AC.T.GT--.

E.viridi .AA.G---.--- .---.---. ---.---. ---.---. ---.---. ---.---.

Eutrept_ G.CGG.-G.- TGG.TCTR-A AC-GG.A.AG -ATG-G.TC- TG-TT.---.

1951 1961 1971 1981 1991 2000

| | | | |

E._graci ATC--GTAAG T-GA-----

Eug._sp.

Astasia_C C.....

E.piscifC..C C.....

Khaw.qua TGTGAT.GGC C.CT.TGGGG AGCCTTGAG GATGTCAAGT GGTCAGGAGC E_anabae

---.---.--- .---.---.

Eacus_co ---CAA.CGT CC.CTA....

E._spiro ---.---.--- .---.---.

E.viridi .G.TGAG... G.C..AAGC. ...ATATGTG TCAACCGAAT CC.....

Eutrept_ ---.---.--- .---.---.

2001 2011 2021 2031 2041 2050

| | | | |

```

E._graci ----- ----TGGGA CTGTT--CGG G----GTG-- AAA-GAT-AC

Eug._sp. ....

Astasia_ ..... .C..... .....T

E.piscif ..... .....G...T Khaw.qua

ATCGAACGGA GACGA..... .CgA.CG... A.....cn.. aa...G...T

E_anabae .....----- -----.--- -...---. ---.---.---

Eacus_co ..... .C...A. ....T

E._spiro ..... .C...A. .... G...G...T

E.viridi ..... .GA..... TC-GCT..A. ....

Eutrept_ .....----- -----.T C..... .TG.....T

2051 2061 2071 2081 2091 2100

| | | | |

E._graci -GG-G-AGCG -CCA-GAG-G TGAAA-TTC- TTAGAT-CGC T-G-CC-AG-

Eug._sp. ....

Astasia_ ..... ..a.....

E.piscif .C..... .....G....

Khaw.qua .T..C..... .g..... C...A.G..

E_anabae .--.-.---- .---.---.- -----.---. -----.--- -.---.---.

Eacus_co .T..C..A.. -..... .....C.... ..A....

E._spiro .T..C..A.. .....C.... ..A....

E.viridi .T..C..A.. .....C.... ..A....

Eutrept_ .T..A.CA.. .....C...C.... ..TG.G..

2101 2111 2121 2131 2141 2150

| | | | |

E._graci ATC---CACT GCA-GC-GAA G-GC---GTT C---TGC--- -AAGTGACAG

Eug._sp. ....

Astasia_ .....

E.piscif .C.....G .....T.

Khaw.qua .C...A... .....GT.

```

E_anabae ---...---- ---,---,--- -,---,--- -,---,---
 Eacus_coA... ..G....AH.T.
 E._spiro .C....A..G ..G..c..G.
 E.viridiAG.G ..G..... ..T..
 Eutrept_A... ..G....A..CA.... .TG.A...T.
 2151 2161 2171 2181 2191 2200

| | | | |

E._graci TCCG--TC-- GAT-CAA-GA -A-T-GAGA- GTTC-GGG-G AG-C-AAA-G
 Eug._sp.
 Astasia_
 E.piscifC.....
 Khaw.qua .T..... ..CT.....
 E_anabae ----,---,--- ---,---,--- -,---,---,--- ---,---,--- -,---,---,---
 Eacus_coT... ..GT.....
 E._spiro .T..... ..T... ..GT.....
 E.viridi .T..A..... ..GCT.....
 Eutrept_ .T..... ..C..... ..GT..... ..G.... 2201
 2211 2221 2231 2241 2250

| | | | |

E._graci ATG-ATCAGA CAC-CGTC-G TA-GTCCGG- CC-AC-TGTA AACGAT-GCC
 Eug._sp.
 Astasia_
 E.piscifC.....
 Khaw.quaA.. -.C... ..TG.....
 E_anabae ---,----- ---,-----,--- --,-----,--- -----,---
 Eacus_coT..AC. -A..... ..C.....
 E._spiroc... c.c....c..c.AC. -G.....c ...T.....
 E.viridi .G..... ..C..... ..A.. -..... ..CG.....
 Eutrept_ T...A..... ..TAC. -..G..... ..T...T.

2251 2261 2271 2281 2291 2300

| | | | |

E._graci -G-GC-C-AG -GCC-TTGGC AGAG---CAA GAATCCTAGA -----

Eug._sp.

Astasia_G.....CTT.CAG

E.piscifT...A G.G....-a. A....AAcAG

Khaw.quaT.C. ...A...ggcG.T CGCATTCCTT-

E_anabae .-.-.-.-. -.-.-.-. -.-.-.-. -.-.-.-. -.-.-.-.

Eacus_co .A....T.Ct ..GT.GGT.A T.GT..G... .CGAT..CAG A.....

E._spiroC. ..T..C...T G.T..... .T.CA.GGA-

E.viridi .A.....A.. ..G....A.C CTCAAACC..

Eutrept_ .A....T.C. ..TG..C..T G.GC.....T A.TCT.CTC. AC.....

2301 2311 2321 2331 2341 2350

| | | | |

E._graci ----- CTCT----- -----G TCAG-GGCCA -CT-CCTC-C

Eug._sp.

Astasia_A .C..G.....-

E.piscifAc .C.....- .a.....

Khaw.qua ...GTTCCCTA .C..G.....- -.CA..TG.C .A..G.C.C.

E_anabae -.-.-.-. -.-.-.-. -.-.-.-. -.-.-.-. -

Eacus_co -.-.-.-.CACCA C...C.C.AT G-CC-.C.T-

E._spiroG.C.....A .C..... T.....C...

E.viridiCC .CGCT.....- --...T....AG...C...

Eutrept_AGGATG .C--.....A ..G..TTT..

2351 2361 2371 2381 2391 2400

| | | | |

E._graci CACAAC-GA- GAAA-TC-C- ACA-GC-CTG TG-GGT-T-C AG-G-GG-G-

Eug._sp.

Astasia_

E.piscif
 Khaw.qua .G.....
 E_anabae -----,--. ----,--,-. --,--,--- --,---,- -,-,--,-.
 Eacus_co -G.....G.C.... -.....A E._spiro
 TG.....C. .-.....C
 E.viridi T.....
 Eutrept_ TG..G.....- .A.....T.A C...A.....
 2401 2411 2421 2431 2441 2450
 | | | | |
 E._graci GAGT-A-CTG TC-GCAA-GG C-T-G-AA-A CTT-AA-AGG A-AT-TGAC-
 Eug._sp.
 Astasia_
 E.piscif
 Khaw.quagT
 E_anabae ----,-,--- --,---,- -,-,-,--,- ---,--,- -,-,---.
 Eacus_cog
 E._spirog.....
 E.viridiGT
 Eutrept_G. ...T.....T. 2451
 2461 2471 2481 2491 2500
 | | | | |
 E._graci G-GAATGGCA -CCACAA-GG CGT--G-G-A GT-A-TGCGG C-TTAA-TTT
 Eug._sp.
 Astasia_
 E.piscif
 Khaw.quag.g.
 E_anabae -,------ ,-----,- --,-,-,- --,-,----- -,-,---.
 Eacus_co
 E._spiro T.....

```

E.viridi .....

Eutrept_ .....

2501 2511 2521 2531 2541 2550

| | | | |

E._graci G-ACTCAACG -CGGG-GAAT GTTA-CCAGG -T-C-AGGA- CGCA-ACTGG

Eug._sp. ....

Astasia_ .....

E.piscif .....

Khaw.qua .....A .....C ..... .A...G...

E_anabae -.----- .----.---- ----.---- .-.-.----. ----.----

Eacus_co .....A ..... ..Tt.g...

E._spiro .....A ..... ..C..... ..TT.G...

E.viridi .....A .....C ..... .A...G..A.

Eutrept_ .....A ..... ....GTC..

2551 2561 2571 2581 2591 2600

| | | | |

E._graci -GA-TTGA-C -AGA-TTGAG AGC-TCT-T- TC-TT-GATC -T-TGTG-GA

Eug._sp. ....

Astasia_ .....

E.piscif .....

Khaw.qua .....G.... ..... .C.....

E_anabae .--.----.- .----.---- ----.----.- --.---.---- .-.----.-

Eacus_co .....G.... .....g.. .....t. ....-C....

E._spiro .....G.... ..... .c..c.... .c.c....t .C.CTc....

E.viridi .....G.... ..... .C.....

Eutrept_ ..... G.T....W. .... .C..C.R...

2601 2611 2621 2631 2641 2650

| | | | |

E._graci C-GG-T-GG- TG-CATGGCC GCT-CC-T-G -ATT-G-GTG GA-GTGA-TT

```

Eug._sp.
 Astasia_
 E.piscif
 Khaw.quac g.....t
 E_anabae -.---.-.---. --.----- ---.---.-.---. ---.---.---.---.---
 Eacus_co T.....
 E._spiro T.....a.gg.....ga...
 E.viridi A.....
 Eutrept_R.....
 2651 2661 2671 2681 2691 2700

| | | | |
 E._graci TGT--C-T-G G-TTGATT-C CG-ATAACG- AGT-GAGACA -TCT-G-CCT
 Eug._sp.
 Astasia_
 E.piscif
 Khaw.quag....
 E_anabae ---.---.-.---. -.-----..CA....
 Eacus_co
 E._spiroT.....
 E.viridi
 Eutrept_C

2701 2711 2721 2731 2741 2750
 | | | | |
 E._graci C-CCACT-AG CCTGAG-GCT C-GCA----- --TTTGGTAG GGTTCGG-C-
 Eug._sp.
 Astasia_G....G..... ..CCT..C..
 E.piscifG....G.....CT..C..

Khaw.qua ..T..A.... ..C..... ..a..... ..T..C..

E_anabaeCCT...AC ...T..... ..-CT.... ..GGACAT.

Eacus_coG.... G.CCC.GT..-C.T.... ..GTC....

E._spiroCC.A.C.. c..c..... ..T.... ..CATCCA..

E.viridiG.... ..G.....C..... ..CT.CC..

Eutrept_ ..TG.A.... T.CCC.GC..T.CAT.... ..--AC.GA.

2751 2761 2771 2781 2791 2800

| | | | |

E._graci TGCT-CG-GT G-GCAG-CCC CCTG-GC--- -----

Eug._sp.

Astasia_ C-...-...CA...- ..G..CGGTG GGGTGTGCGT TTCGGCGTGC

E.piscif CT...-....T GGGT.T.... ..

Khaw.qua cTTG.G..T. ..C.T..T.A ..CA.CTTTT GGGT.TGCGC ACGCAAGTGT

E_anabae G.GCTG.TCC CTTTCTGGGG ..C.CAAGCA TCCCATTCCC TTTC.....

Eacus_co -----... ..-...-...-...-...-... .. E._spiro

G....G..CC AC..G..gg. TTGCC-.C.. ..

E.viridi A.TG.AA.CG ...TCC..TG TTGT..AGAC CACTGGGGTT CGTCCCCTCT Eutrept_

ACAC.-T.TG C.C.T..TGG GTC....TTG CCGGT.CCCA CC.....

2801 2811 2821 2831 2841 2850

| | | | |

E._graci ----- AA-CAGGGGG -AGA-TGT-A

Eug._sp.

Astasia_ ACTTT..... .. CC..CC.... ..G

E.piscif-GCCC... ..G

Khaw.qua GTGATCCAGA ATGTTGTGGT GG..... --...C.T. .T.C.GCC.C

E_anabae-TT....AG Eacus_co

TGGGAAGAGg CGGTTC.CC. TCCTTG.... E._spiroGT..T.. C---...C.-

E.viridi-G.G...ATC .GAC.AC-..

```

Eutrept_ .....G..... --,----- ,---,.,GGC

2851 2861 2871 2881 2891 2900

| | | | |

E._graci CC-GGTG-CA TGCTCCCGA- GAGCCTC-C- AG-TTCA--- -----
Eug._sp. ....
Astasia_ ...A.C-.GG G.....
E.piscif .....-T G.....
Khaw.qua AT..TCT.TT Gg.....G. .A.GATCATC A.....
E_anabae --....K.GG -C..ST---. ...--.T... .A.-C..CTR AGGCCAGGG
Eacus_co ..CTCG.CGG -.A..T... ..G--.... ..TGGT.TA. ....
E._spiro --,----.GG .CTG..T... ..GT...T. G.G-ATCA.. ....
E.viridi ..CC.CT.GG C..G..... ..C.... ....-TCCCA AAT.....
Eutrept_ T.GT.CAT.. CC.GT.T... ..TGC..G. G.--T.TGC T.....

2901 2911 2921 2931 2941 2950

| | | | |

E._graci ----- ---GCT-TCT CT-GAGG-TG CT---GTGTC C---GCCACA
Eug._sp. ....
Astasia_ ....
E.piscif .....T...
Khaw.qua .....T .....Tg.C
E_anabae TTT.....TTC..
Eacus_co .....A .....C
E._spiro .....A .....T..C
E.viridi .....A .....G.C

Eutrept_ .....CG..... .CG..CGCAT G...T..G.C 2951
2961 2971 2981 2991 3000

| | | | |

E._graci AA---GGGC- --AT---GCA TGCT-AGA-G CC-A-AC-AG -C-A-GGTC-
Eug._sp. ....

```

Astasia_C.....a.a.....
 E.piscifC.....
 Khaw.quaA...C.....
 E_anabaeA.....
 Eacus_coT...C.....
 E._spiroT.....
 E.viridiT.....
 Eutrept_ ..G..T.CG. ..CG...-.. A..-..... G.....
 3001 3011 3021 3031 3041 3050

| | | | |

E._graci TGTG-ATGCT CC-CAGAT-G -T-CCTGG-G CC-GC-A-CG CG-C-ACT-A
 Eug._sp.
 Astasia_ ...t.....
 E.piscif
 Khaw.quaT.....
 E_anabae
 Eacus_co
 E._spiro ...n.....g.....
 E.viridi
 Eutrept_T.....T.....
 3051 3061 3071 3081 3091 3100

| | | | |

E._graci -C-ATT-GTC ACAG-TGA-A GG-TGTCG-A CAT-GCCCAC --TCCG----
 Eug._sp.
 Astasia_GTC ..A.....
 E.piscifCTTT ..A.....T.....
 Khaw.quaA. T.G.....-TC .T..T..AG.ACCAGC
 E_anabaeA. T.....GG-.. --CT..AT. ---CCA..
 Eacus_coGT C.....T.....CTG ..-..T.C- .C..T.T...

E._spiroG. C..... T....CT-.C A.C...TTG- ..CT..T...

E.viridiC...AT CG..... ...A...CT. GTG.C.TGCT ..CTGT....

Eutrept_C...GT C..... CA.A.C.TT- ---..GTG--CTTCC

3101 3111 3121 3131 3141 3150

| | | | |

E._graci -----G TGGGC----- -----C CTG----GCC T--GAAG-AG

Eug._sp.

Astasia_T..

E.piscifT..

Khaw.qua TTC..... ..T.AGC... .. .TT. A.....A.T.

E_anabae AT..AG....CA. A.....T.

Eacus_co GA.--..... G.....C.T G.....T.

E._spiroC AA.A-..... ..T....C.T G.....Tg

E.viridiC A.RC-.....G.....T.

Eutrept_ ATGGGA...C C.T--.....C.. A.....T.

3151 3161 3171 3181 3191 3200

| | | | |

E._graci GCTGGG--AA AT-CCT-GCA AGC-CT-GTG AC-----GTA CTG-----

Eug._sp.

Astasia_ .A.....

E.piscif .A.....A G.....

Khaw.qua AA.....C.. .AT..C..A. C.....G E_anabae

TG.....A. CT.....

Eacus_co .G..... .C.....G. GC-.....C TGACC.....

E._spiro .G.Tgg.... .C.....A. GC-.....Gc C.TTGCC... ..

E.viridiC.....A.. ..T....GA T.....G ..

Eutrept_ .G.....C. .C..T..TGC ..-..... .ATGC....G T.T.....

3201 3211 3221 3231 3241 3250

| | | | |

E._graci ----GGGAT- AG-A----- TGG--TTGCA ACTGT--CTG -----C-

Eug._sp.

Astasia_A.....

E.piscifA.....

Khaw.quaA.....

E_anabaeA.....C

Eacus_coA.....

E._spiroA.....

E.viridiA....T.

Eutrept_T.....-C.....A.C.-...T.

3251 3261 3271 3281 3291 3300

| | | | |

E._graci CT---TGAAC GTGGA-ATGC C-TAGTAT-G -C-CTGGG-T CAT-CAG-CC

Eug._sp.

Astasia_A.....

E.piscifA.....

Khaw.quaA.....

E_anabaeA.....G.T....

Eacus_coA.....A..G.....

E._spiro c.....A.....A..GCA....

E.viridiG..G.T....C.....

Eutrept_ .A.....AA.....G.....T.GCAC...T

3301 3311 3321 3331 3341 3350

| | | | |

E._graci CAG-A-C-CG A-TTGTGTCC C-TGCCATTT GT-A-CA-CA CCGCCCGTCG

Eug._sp.

Astasia_A.....

```

E.piscif .....
Khaw.qua .....A. ..C.....
E_anabae A.C.....A. ..A..C.... .....T.
Eacus_co ..C.....T. ....
E._spiro TGC.....T. ....
E.viridi A.C.....
Eutrept_ TGC.....A. ....A..... A.....A...
3351 3361 3371 3381 3391 3400

| | | | |

E._graci T-TGCT-A-C C-GA-T-GGT GGCT-GG-AT A-GA-GTC-A TCAGGA-GGT
Eug._sp. ....
Astasia_ ....
E.piscif .....
Khaw.qua .....R.. .....A. A.....
E_anabae .....T. ....C .....G... .Y.K....C
Eacus_co .....A.....C.....CG
E._spiro .....C AT.g.....C... ..A
E.viridi .....A. C.....AC...
Eutrept_ ..... T.....C A.AA.T.... ..A...C..C .....AC. 3401
3411 3421 3431 3441 3450

| | | | |

E._graci T-G--T-GGA -G-GC-GGC- CTCT-----C GG-----GG -C-AG-TAGA
Eug._sp. ....
Astasia_ C....G...C .C.....G. -..C..... TCGcgG..AC .....
E.piscif C.-.-.--G ...A..CA.. .A..... TCGGGTG.C. .T.GC.GTT-
Khaw.qua C.A..C.g.T .C.A..-A.T TG.CAAA..T TCTT....TT .T.G..C...
E_anabae C.AGCCT.TG G.T.Tt.TYA aGG.GGG.T. CcTtCCCACC ATGGgT.CC.
Eacus_co C.T..-.-. -..c.CA.T ..... C-.....- -.-.-.-
E._spiro C.C..-T.TT .-.-.-.- -C.CCCC..- --.....T .-G.G----
E.viridi C.A..-..C .C.AG.CC.. ..-.-.-.- --.....- .T.G-..C..

```

Eutrept_ C.A..A..CT ...AT.T.G. YA.....T .TG.ACA.TC .A..T.GTTT

3451 3461 3471 3481 3491 3500

| | | | |

E._graci -G-C-A-TCC C-AGCC-G-- AAA-TTG-GA C-GA-TGT-C C-AGCCACTA

Eug._sp.

Astasia_ ..TG.G.C.AA..... ..TG...

E.piscif .-.-.-.-. ...A..... ..A..... ..TG...

Khaw.qua ...A.-.C..T.... c....TGT..

E_anabae AaCAACAC.. .A...A..cc ...C.....T ..A.....T c.....A..

Eacus_co G-T-C-.--- -AG..T.A..G ..A..... ..T.....

E._spiro .-AA.-.-. .AG..T.g..G ..A..... ..CCGTG...

E.viridi-.-.AT....G T.....C..TG....

Eutrept_ ..TN.-.-.--- -.-AT.... G..T....GT ..T.TTG...

3501 3511 3521 3531 3541 3550

| | | | |

E._graci -GA-GG-A-A GCA-AAAGTC GTAACAAGG- TTGCT-G-TA GG-----TGA

Eug._sp.N.....

..... .g.....

Astasia_

E.piscifgc..... ..T.

Khaw.quatgct.g.ta gg.....tga

E_anabaek.. ..

Eacus_co tt

E._spiroT.C.C..TT...

E.viridiaa... ..

Eutrept_ gga.aaagtt gt

Figure 1a. Although both organisms belong to *Euglena anabaena*, there is a significant size difference. This is due to culture and/or light conditions.



Figure 1b. An illustration of the shield-shaped chloroplast with a paramylon cap in *Euglena anabaena*.

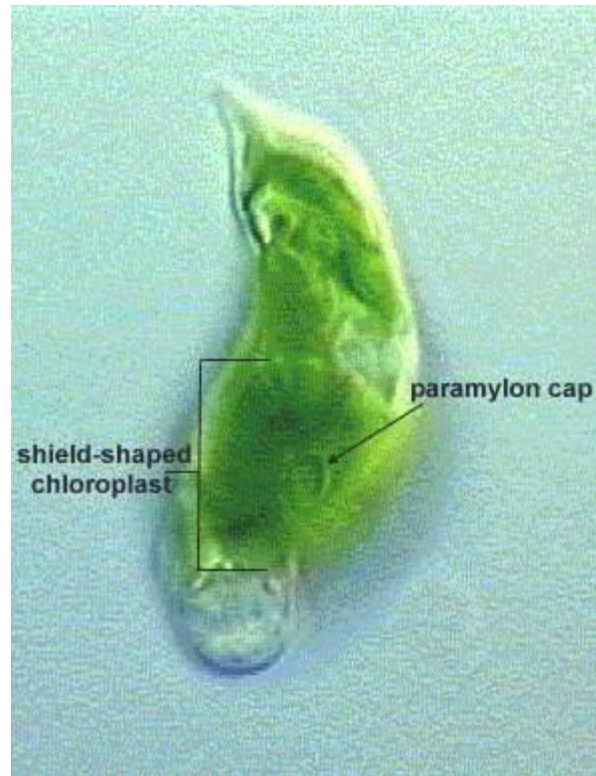


Figure 1c. The emergent flagellum(a) and eyespot (b) of *Euglena anabaena*.

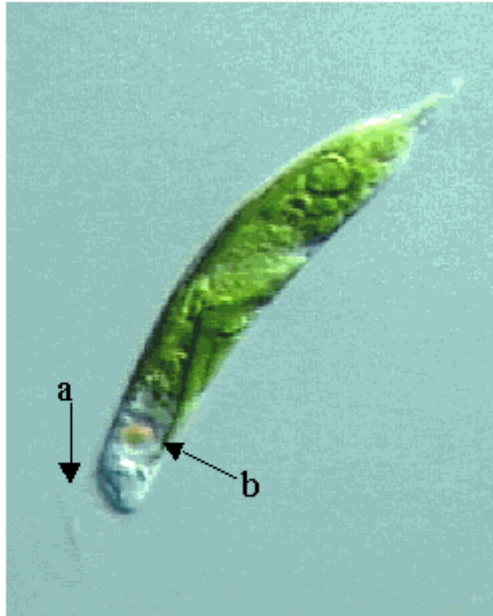


Figure 2. The secondary structure of the 18S SSU rDNA of *Euglena gracilis*

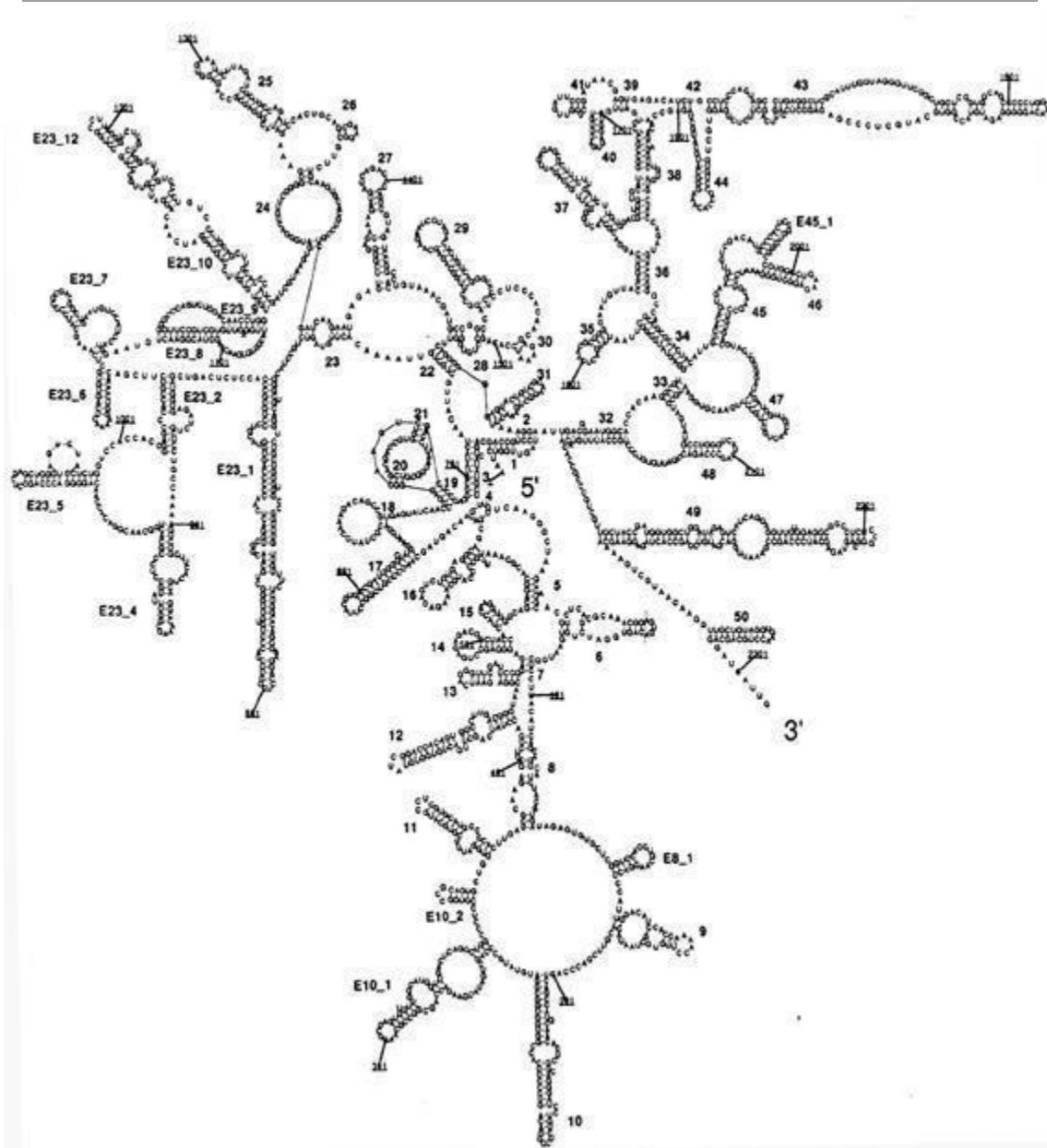
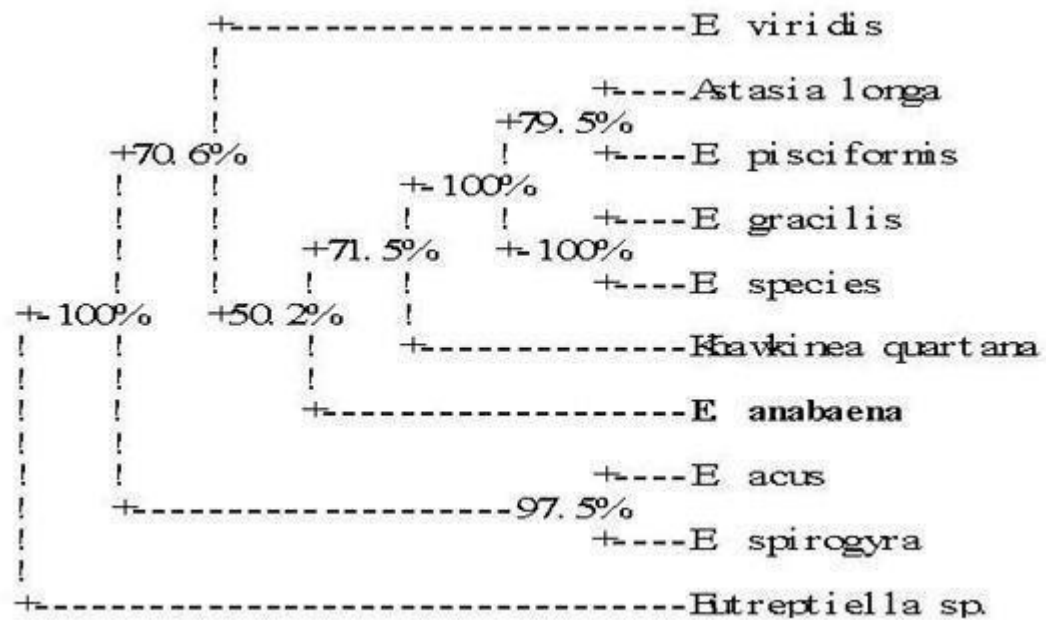


Figure 3. The Majority Rule tree created from 500 bootstrap values. CONSENSUS TREE: The numbers at the forks indicate the number of times the group consisting of the species which are to the right of that fork occurred among the trees, out of 500 trees.



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