Systematics of primary osmotrophic euglenids: a molecular approach to the phylogeny of Distigma and Astasia (Euglenozoa)

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Nuclear-encoded SSU rRNA genes from nine strains of Distigma and three strains of Astasia were sequenced and analysed phylogenetically with maximum-likelihood and maximum-parsimony methods. It could be demonstrated that the genus Distigma is paraphyletic, consisting of two distinct clades: one comprises four strains of the type species, Distigma proteus, and the other includes four strains of Distigma curvatum, Distigma gracile, Distigma sennii and Distigma elegans. These findings are well corroborated by morphological characteristics. The investigated species of Astasia are closely related to members of the Rhabdomonadida, thus rendering the genus Astasia polyphyletic, with Astasia longa branching within the phototrophs. All of the species investigated cluster in a well-supported group of primary osmotrophic euglenids that are not derived from photosynthetic ancestors. The recovered clades are characterized by their sequence diversity. After different evolutionary rates among lineages had been determined, a modified slow–fast approach was used to differentiate phylogenetic signal from noise. Finally, a revised systematic scheme based on phylogenetic relationships is suggested to render euglenid taxonomy more transparent: primary osmotrophic euglenids are classified as Aphagea, and members of the D. curvatum group are transferred into the new subgenus Parvonema.

INTRODUCTION

Euglenids are a diverse group of protists that include phototrophic, phagotrophic and osmotrophic species. Phagotrophs are the base of the euglenid tree and probably represent the plesiomorphic mode of nutrition. Phototrophic forms emerged by means of secondary endocytobiosis: the chloroplast of an ingested green alga was established as the euglenid plastid. The origin of osmotrophic euglenids has been debated for a long time (e.g. Leedale, 1967, 1978; Dawson & Walne, 1994), as most of them lack taxonomically useful characteristics such as ingestion apparatuses or plastids. Use of molecular data (Linton et al., 1999, 2000; Preisfeld et al., 2000, 2001) and cladistic analyses of morphological characteristics of the euglenid pellicle (Leander et al., 2001) has revealed that only a fraction of the osmotrophic euglenids derived from phototrophic ancestors, while others originated from phagotrophs.

Members of the genus Distigma Ehrenberg emend. Pringsheim are characterized by two emergent flagella, a pronounced metaboly and the lack of ingestion devices, leading to a mode of nutrition supposed to be osmotrophic (Pringsheim, 1936, 1942; Skuja, 1948, 1956; Christen, 1959, 1962). This was supported by immunocytochemical results (Yamaguchi & Anderson, 1994), which located reaction products of acid phosphatase in small vesicles near the reservoir and canal region of Distigma proteus. This led to the idea of pinocytotic uptake of nutrients in this area. In his first description, Ehrenberg (1838) noticed two dark spots close to the base of the flagella, which he misinterpreted as eyespots, hence the name ‘Di-stigma’. It is now known that Distigma possesses neither an eyespot nor plastids (Yamaguchi & Anderson, 1994). De Fromentel (1874) was the first to propose a derivation of Distigma from the phototrophic genera Eutreptia and Eutreptiella by the loss of plastids, which is analogous to the proposed evolution of Astasia longa from Euglena gracilis-like ancestors (Siemeister & Hachtel, 1989).

Angeler and colleagues pointed out several features that are taxonomically important for the genus Distigma (Angeler, 1999, 2000; Angeler et al., 1999). These include...
the proportional length of the ventral flagellum in comparison with the dorsal one, the appearance of nuclear endosomes and the ultrastructure of the pellicle. D. proteus was found to possess symbiotic bacteria in its cytoplasm; because they were obviously not digested, it could be deduced that Distigma is derived from phagotrophic ancestors (Yamaguchi & Anderson, 1994; Angeler et al., 1999).

Early molecular investigations showed that D. proteus and Distigma curvatum are closely related to members of the Rhabdomonadida, thus constituting a well-supported clade composed of primary osmotrophic euglenids (Preisfeld et al., 2000, 2001). Furthermore, two Astasia species were found to branch within the primary osmotrophs rather than being closely related to the phototrophs (Linton et al., 1999, 2000; Müllner et al., 2001). Cladistic analyses of morphological characteristics confirmed the monophyly of primary osmotrophs and their divergence from phagotrophic ancestors with a flexible pellicle capable of euglenid movement (Leander et al., 2001).

The aim of this study was to investigate the phylogeny of Distigma, based on an SSU rDNA dataset. For this purpose, 12 new sequences from various Distigma and Astasia species were added to published sequences and the resulting dataset was critically analysed. Some taxonomically important morphological features were compared with the results based on molecular data, leading to the suggestion of a revised euglenid taxonomy.

METHODS

Organisms, DNA extraction, amplification and sequencing. Cultures of Distigma and Astasia species were obtained from the Culture Collection of Algae at the University of Göttingen, Germany (SAG), and the Culture Collection of Algae and Protozoa (CCAP), Ambleside, UK. The organisms were grown according to the culture-collection protocols. Total cell DNA was extracted using the DNeasy plant DNA purification kit (Qiagen). Amplification of nearly complete SSU rDNA was performed with universal eukaryotic primers following by the PCR protocols described previously (Preisfeld et al., 2000). PCR products were cloned into the pCR 2.1 vector using the TOPO TA cloning kit (Invitrogen). Sequencing was conducted with standard M13 primers as well as additional primers in both directions (primer walking).

Phylogenetic analyses. Twelve new sequences from the genera Distigma and Astasia were aligned with published euglenozoan sequences. Initial alignments with CLUSTAL X gave only a rough estimate of positional homology. Consequently, the alignment was corrected manually, taking into account secondary-structure information from various organisms. Only unambiguously homologous positions were retained for phylogenetic inference.

Prior to maximum-likelihood (ML) tree inference, the best-fit model of sequence evolution was determined using MODELTEST 3.06 (Posada & Crandall, 1998). ML tree inference was undertaken using PAUP*, version 4.0b8 (Swofford, 1998); heuristic searches applying random addition of sequences and 10 replications have been conducted. ML bootstrapping was performed using a neighbour-joining tree as the starting topology and running 7500 rearrangements on each of the 100 replicates. Maximum-parsimony (MP) bootstrapping was done with random addition of sequences and 10 replications on each of the 500 bootstrap pseudosamples.

The Shimodaira–Hasegawa test, as implemented in PAUP*, was used to test for significant differences in likelihood among the ML tree and constrained topologies.

To characterize several monophyletic groups, revealed after phylogenetic analyses, the mean sequence diversity and standard deviation for selected groups were plotted. For this purpose, the pairwise ML distances, according to the optimal tree under the selected model of sequence evolution, were used.

A likelihood ratio test was performed with MODELTEST 3.06 to investigate the appropriateness of a molecular clock assumption. Therefore, the score of the optimal ML tree constrained to a molecular clock was calculated and compared with the score of the best tree without a clock assumption.

A relative-rate test implemented in PHYLRTEST 2.0 (Kumar, 1996) was used to test the hypothesis of rate constancy among lineages. For distance calculation, Kimura’s two-parameter model of sequence evolution (Kimura, 1980) with gamma distribution (alpha = 0.8) was used. The sequences were arranged into groups and thus, different lineages could be compared, instead of single sequences.

In an attempt to differentiate phylogenetic signal from noise, and to deal with a non-stationary evolutionary rate among sites and taxa, a modified slow–fast approach according to Brinkmann & Philippe (1999) was performed. On the basis of an MP tree, the characteristics were grouped into categories according to the number of tree steps required at each position. This procedure allows for analyses of datasets that contain only a subset of the variable positions and therefore different proportions of homoplasy. Decay support for monophyletic groups found in parsimony analyses was determined with PAUP*, using the clad constraint method described by Morgan (1997).

RESULTS

SSU rRNA-encoding regions from 12 euglenid species of the genera Distigma and Astasia were sequenced and analysed. The sequences were added to a euglenozoan dataset comprising euglenids, diplomemids, kinetoplastids and outgroups. The alignment contained 1141 characters, of which 617 were parsimony-informative.

After ML tree inference, the newly analysed sequences cluster in a well-supported clade of primary osmotrophic euglenids, including the genus Distigma, some species of the genus Astasia and the Rhabdomonadida (Fig. 1). The primary osmotrophs form the sister taxon to the phototrophs (including A. longa) and the phagotrophs Peranema trichophorum. Monophyly of euglenids, including the phagotroph Petalomonas cantusygni as first descendant, is weakly supported.

Within the primary osmotrophic euglenids, several subgroups can be distinguished. The genus Distigma appears paraphyletic as two well-supported clades. One of them, referred to as the D. proteus group, forms the base of the osmotrophic subtree containing three strains of the type species D. proteus Ehrenberg and Distigma gracile SAG 216.80. The other clade, called the D. curvatum group
according to the first species described, contains four strains of *D. curvatum*, which appear to be paraphyletic, intermingled with *D. gracile* CCAP 1216/2. *Distigma elegans* and *Distigma sennii* are at the base of this subtree; there is no evidence for a close relationship between these two species. Further interrelationships within the *D. curvatum* group could not be resolved. It must be emphasized that the analysed strains assigned to *D. gracile* belong to two independent clades.

The closest relatives of the *D. curvatum* group are represented by a strongly supported clade consisting of the monophyletic Rhabdomonadida and five strains of the genus *Astasia*, which do not form a monophyletic group.

By constraining the ML analyses, several user-defined trees were generated to address the significance of some hypotheses regarding the phylogeny of osmotrophic euglenids by Shimodaira–Hasegawa tests (Table 1). Constraint trees showing the monophyly of the Eutreptiina sensu Leedale (1967), combining the phototrophic genera *Eutreptia* and *Eutreptiella* as well as the osmotrophic genus *Distigma*, are significantly worse. Similarly, a monophyletic genus *Astasia* is rejected. The monophyly of the four analysed strains of *D. curvatum* is significantly dismissed. Although the sister-group relationship between the *Astasia torta* group and the Rhabdomonadida is supported with 100 % bootstrapping, the Shimodaira–Hasegawa test does not significantly reject the monophyly of the primary osmotrophic *Astasia* species.

In order to characterize the groups revealed within the primary osmotrophic clade, the pairwise ML distances have been plotted (Fig. 2). On the basis of SSU rDNA sequence data, the Euglenozoa, and euglenids in particular, are extremely diverse taxa. The distances between groups are

### Table 1. Shimodaira–Hasegawa tests comparing the ML tree shown in Fig. 1 with user-defined trees

<table>
<thead>
<tr>
<th>Tree topology</th>
<th>$-\ln L^*$</th>
<th>$\Delta -\ln L$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML tree (Fig. 1)</td>
<td>15101·68</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Eutreptiina monophyletic†</td>
<td>15241·35</td>
<td>139·67</td>
<td>$&lt;0·001¥$</td>
</tr>
<tr>
<td><em>D. curvatum</em> strains monophyletic</td>
<td>15249·32</td>
<td>147·64</td>
<td>$&lt;0·001¥$</td>
</tr>
<tr>
<td>Genus <em>Astasia</em> monophyletic</td>
<td>15561·35</td>
<td>459·67</td>
<td>$&lt;0·001¥$</td>
</tr>
<tr>
<td>Primary osmotrophic <em>Astasia</em> monophyletic</td>
<td>15150·80</td>
<td>49·11</td>
<td>0·113</td>
</tr>
</tbody>
</table>

*Likelihood of the best tree.
†Difference in $-\ln$ likelihood between the best tree (Fig. 1) and the user-defined trees.
‡Eutreptiina sensu Leedale (1967), comprising the phototrophic genera *Eutreptia* and *Eutreptiella* and the osmotrophic genus *Distigma*.
§The user-defined tree was significantly worse than the best tree at $P<0·05$. 

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extraordinarily large (data not shown), whereas some groups exhibit moderate or low internal diversity. The primary osmotrophs, although well supported in phylogenetic analyses, are a genetically diverse taxon. On average, the paraphyletic genus *Distigma* shows a high degree of genetic diversity, but the standard deviation indicates a broad range of pairwise distances. Internally, the two *Distigma* groups are very homogeneous, leading to the conclusion that the high degree of diversity is due to pairwise distances between the two groups. Essentially, the four strains of the *D. proteus* group are almost identical, whereas the four *D. curvatum* strains are clearly different. The sequences of *D. curvatum* SAG 1216-1b and *D. gracile* CCAP 1216/2 are in complete agreement. The sequence divergence of the primary osmotrophic *Astasia* species and the Rhabdomonadida (identified as AC+AT+R) in Fig. 2) is comparable to that of phototrophic euglenids. The Rhabdomonadida are characterized by moderate sequence divergence.

Since the sequence divergence characterizes the primary osmotrophic euglenids as a genetically heterogeneous taxon consisting of homogeneous subgroups, an examination of the evolutionary rate within the dataset is necessary. A likelihood-ratio test with MODELTEST reveals that the assumption of a molecular clock could be rejected at the 1% level (data not shown). Subsequently, a relative-rate test was applied to investigate which lineages evolve at different rates. The results indicate that the sequences of the primary osmotrophic clade exhibit significantly different evolutionary rates compared with all other lineages (phototrophic euglenids, *Peranema trichophorum*, *Petalomonas cantuscygni*, diplonemids and kinetoplastids) within the dataset. In contrast, the subgroups within the primary osmotrophs do not differ significantly in evolutionary rate, with the exception of *Astasia curvata*.

The marked sequence diversity and the differences in evolutionary rate hint at the possibility that the sequences of the primary osmotrophs suffer from long-branch-attraction phenomena. In an attempt to deal with rate variation among lineages and sites and to distinguish noise from phylogenetic signal, a modified slow–fast approach was undertaken. The new datasets contained different categories of variable sequence positions measured as tree steps in the most parsimonious tree. Positions that change once over the tree topology are completely free of homoplasy and support well-known groups such as phototrophic euglenids, Rhabdomonadida, diplonemids and kinetoplastids (Fig. 3a). This dataset also supports the monophyly of the primary osmotrophic euglenids as well as most of the subgroups recognized before. As more variable sequence positions were added, the consistency index decreased and the signal-to-noise ratio was lowered. During the slow–fast procedure, the Bremer support for the primary osmotrophic clade and most of its subtrees increases continuously (Fig. 3b). Some clades revealed by ML analysis, such as the *A. torta* group or the entire euglenid group, are not supported at any step in the slow–fast approach.

Taking these results into consideration, the identity and composition of primary osmotrophic euglenids could be regarded as reliable and not a result of a long-branch-attraction artefact. Thus, the primary osmotrophs are to
be regarded as a well-founded clade within the euglenids, consisting of equally well-corraborated subgroups.

**Signature sequences**

In addition to high bootstrap support and low genetic diversity implied by the SSU rDNA data, the *D. curvatum* group can be characterized by highly conserved sequence motifs (Fig. 4). These signature sequences are located within variable regions V2 and V4, surrounded by hyper-variable areas for which no homologous positions could be identified either within this group or among euglenids. Although these motifs are conserved among the *D. curvatum* group, no homologous positions could be found in *D. proteus* or in the remaining members of the Euglenozoa. These complex nucleotide patterns can be considered an antapomorphy of the *D. curvatum* group, supporting their monophyly and their distinctiveness from *D. proteus*.

**DISCUSSION**

**The genus Distigma**

In this contribution, based on SSU rDNA data, we identify the genus *Distigma* as being paraphyletic, comprising two distinctly separated groups. The *D. proteus* group consists of four strains with nearly identical SSU rDNA sequences. In agreement with findings of Angeler (1999), based on morphological and ultrastructural data, this leads to the conclusion that all of them represent *D. proteus* Ehrenberg 1838. Angeler transferred *Distigma levis* SAG 1216-6 and *Distigma pringsheimii* SAG 1216-7 (Schlösser, 1994) to *D. proteus*, which is corroborated by our ML and sequence-divergence analyses. The same applies to the *D. gracile* strain investigated, SAG 216.80, which Angeler supposed to be mislabelled. In summary, the *D. proteus* group is composed of one species represented by four strains.

The *D. curvatum* group includes four strains of *D. curvatum*, *D. gracile* strain CCAP 1216/2, *D. elegans* and *D. sennii*. In contrast to the *D. proteus* strains, the ML tree topology and marked genetic distances indicate that *D. curvatum* comprises more than a single species. For instance, the species descriptions of *D. curvatum* (Pringsheim, 1936), *D. gracile* (Pringsheim, 1942) and *Distigma glabrum* (Christen, 1958; formerly assigned to *D. curvatum* SAG 1216-5) are very similar, as already stated by the respective authors. As distinctive features, they identified cell size and shape, characteristics which are to be considered insufficient for species diagnoses. Further morphological and ultrastructural investigations will be necessary to evaluate the species status of these strains and the validity of some of the diagnoses. Although some authors have proposed a close relationship to *D. proteus* (e.g., Christen, 1959), both *D. sennii* and *D. elegans* branch unambiguously within the *D. curvatum* group according to our molecular analysis. Moreover, *D. sennii* and *D. elegans* are not as closely related as postulated previously (Angeler et al., 1999; Angeler, 2000). These species are distinguished by different numbers of subpellicular microtubules, which may be responsible for the different capacity for metabolic movement (Angeler et al., 1999). However, a well-supported branching order within the *D. curvatum* group could not be achieved.

Morphologically, the genus *Distigma* is based mainly on plesiomorphic characteristics such as two emergent flagella, a flexible pellicle and a lack of chloroplasts. The splitting of *Distigma* into two distinct groups in molecular analyses (Preisfeld et al., 2000, 2001) is substantiated by morphological and ultrastructural characteristics: *D. proteus* possesses two flagella of equal thickness, the shorter flagellum reaching one-third of the body length. The nucleus contains up to three nucleoli, pellicle strips appear sigmoidal in transverse section and the surface is not covered by organic deposits (Angeler et al., 1999; Leander et al., 2001). In contrast, members of the *D. curvatum* group show a strongly reduced ventral flagellum and only one nucleolus per nucleus, and the pellicle is covered by a marked layer of organic deposit. Hence, we propose to create a new subgenus, *Parvonema* subgen. nov., comprising the species of the *D. curvatum* group.

**The genus Astasia**

The molecular data place the species of *Astasia* investigated in close affinity with the Rhabdomonadida, as shown

![Fig. 4. Signature sequences characterizing the *D. curvatum* group. Positions of signature sequences are given by reference to the SSU rDNA sequence of *D. sennii*. Accession numbers of sequences are given. Question marks in the sequence of *D. proteus* indicate that no homologous areas could be identified within this sequence. Signatures 1 and 2 are located in V2 and signature 3 in V4.](image)
Previously by Müllner et al. (2001) using a smaller dataset. In contrast to A. longa (Linton et al., 1999, 2000), these species are not derived from phototrophic ancestors and thus belong to the primary osmotrophs. This ‘diphylectic origin’ of the genus Astasia was discussed previously by Leedale (1978). One reason for the polyphyly of the genus Astasia may be the imprecise circumscription, as it includes colourless uniflagellate euglenoids capable of shape change (Pringsheim, 1942), neglecting the phylogenetic origin of osmotrophy. It could be speculated that additional species of Astasia will also show a close relationship to the Rhabdomonadida. Consequently, we suggest the transfer of secondarily osmotrophic Astasia species, such as A. longa, to Euglena. Since only three primary osmotrophic Astasia species were studied molecularly and no ultrastructural data are available, we informally term this assemblage ‘Euastasia’, following Müllner et al. (2001). Furthermore, the unavailability of the type species Astasia limpida Dujardin hinders taxonomic revision of the genus.

Primary osmotrophic euglenids

The euglenid primary osmotrophic clade has been recognized since molecular SSU rDNA data were used for phylogenetic reconstruction (Preisfeld et al., 2000, 2001; Frantz et al., 2000; Müllner et al., 2001). Our analyses corroborate the hypothesis that these organisms form an independent lineage within the euglenids without any phototrophic ancestor. Instead, primary osmotrophic euglenids are likely to be derived from phagotrophic ancestors (Leander et al., 2001). Correspondingly, the occurrence of cytoplasmic endobacteria in D. proteus (Yamaguchi & Anderson, 1994) could be ascribed to undigested prey of a phagotrophic euglenid.

A common feature of primary osmotrophs is an accelerated evolutionary rate, detectable in SSU rDNA sequences, accompanied by drastically enlarged genes encoding the small ribosomal subunit (Busse & Preisfeld, 2002b). As could be shown by several approaches, this characteristic does not interfere with phylogenetic reconstruction by masking phylogenetic signal or long-branch-attraction effects.

Morphologically, the primary osmotrophs are combined because of negative characteristics such as the absence of plastids and ingestion devices. A putative synapomorphy might be the possession of split-ringed structures around the canal, as described for D. proteus (Leander et al., 2001), and which potentially represents a homologue of the scroll of the Rhabdomonadida (Leedale, 1967; Leedale & Hibberd, 1974). Additionally, primary osmotrophs share a common habitat, as all species were found in freshwater (Huber-Pestalozzi, 1955). On the basis of morphological and ultrastructural data, some noticeable evolutionary trends within this clade can be revealed (Leander et al., 2001). (i) The length of the ventral flagellum is continuously reduced: D. proteus possesses a ventral flagellum that reaches nearly one-third of the body length. In the D. curvatum group, it is further reduced to a short stub in D. sennii, whereas no ventral flagellum is emergent in A. curvata, A. torta or the Rhabdomonadida. (ii) The rigidity of the pellicle increases: whereas the pellicle of D. proteus is flexible, D. sennii is only moderately capable of euglenid metaboly. All members of the Rhabdomonadida are completely rigid, with a massive epilamellar layer. This is correlated with a change in the appearance of the pellicle strips: they change from sigmoidal in D. proteus to flat in the Rhabdomonadida, in which they appear to be fused.

Euglenid taxonomy

Since the results presented have an impact on the understanding of euglenid phylogeny, we suggest a revision of parts of the existing taxonomic scheme based on the identification and naming of monophyletic taxa.

Several studies have shown the unambiguous monophyly of the primary osmotrophic euglenids (Busse & Preisfeld, 2002a; Preisfeld et al., 2000, 2001; Müllner et al., 2001). Consequently, we redefine the Aphagea [International Code of Zoological Nomenclature (ICZN); Aphagophycidae International Code of Botanical Nomenclature (ICBN)] of Cavalier-Smith (1993) to include solely primary osmotrophic euglenids that lack any vestiges of ingestion devices. Phototrophic euglenids, which have been a part of Cavalier-Smith’s Aphagea, have been classified as Euglena (ICZN; Euglenophycidae ICBN).

The suborder Eutreptiina Leedale (ICZN; Eutreptiales ICBN) has been emended, now comprising exclusively the phototrophic genera with two or more emergent flagella: Eutreptia, Eutreptiella and Tetreutreptia.

The Rhabdomonadida and the primary osmotrophic Astasia species have been included within the Rhabdomonadida (ICZN; Rhabdomonadidae ICBN). Although several distinctive features of the D. curvatum group could be revealed, no unambiguous synapomorphy could be shown to link this group to the Rhabdomonadida, thus supporting the paraphyly of Distigma. Consequently, species of the D. curvatum group are transferred into the new subgenus Parvonema.

Diagnoses

Diagnoses for new taxa are given according to the ICZN and the ICBN, following the approaches of several authors regarding the ambigeregal taxonomic status of protist taxa such as the euglenids (Patterson & Larsen, 1991, 1992; Larsen & Patterson, 1990; Novarino & Lucas, 1993, 1995).

Diagnosis of Aphagea Cavalier-Smith 1993

emend. Busse et Preisfeld (ICZN); Aphagophycidae subclasis nov. (ICBN)

Osmotrophic euglenids lacking photosensory apparatus and plastids; one or two emergent flagella; no ingestion apparatus. Composition: Rhabdomonadida Busse et Preisfeld...
and Distigma Ehrenberg (ICZN); Rhabdomonadidae Busse et Preisfeld and Distigma Ehrenberg (ICBN).

**Latin diagnosis of Aphagophyceidae subclassis nov.**

_Euglenophyceae osmotrophicae; sine apparatu photosensorico; sine plastis; cum uno vel duobus flagellis emergentibus; sine apparatu ingestione._

**Diagnosis of Rhabdomonadida subclassis nov. (ICZN); Rhabdomonadidae superord. nov. (ICBN)**

Osmotrophic euglenids with one emergent flagellum; pellicle rigid or flexible. Composition: Rhabdomonadidae Leedale 1967 and Astasia (ICZN); Rhabdomonadales Leedale 1967 and Astasia (ICBN).

**Latin diagnosis of Rhabdomonadidae superord. nov.**

_Euglenophyceae osmotrophicae cum uno flagello emergente; pellicula rigida vel metabolica._

**Diagnosis of Parvonema subgen. nov. (ICZN); Parvonema subgen. nov. (ICBN)**

Primary osmotrophic euglenids with two emergent flagella. Ventral flagellum more reduced than in _D. proteus_ Ehrenberg; pellicle strips flat with deposited organic material; no endobacteria, one nucleolus per nucleus. Type species: _Distigma sennii_ Pringsheim 1942.

**Latin diagnosis of Parvonema subgen. nov.**

_Euglenophyceae osmotrophicae; flagella duo, ambo ex canale emergentia; flagellum ventrale magis reductum quam apud Distigma proteus Ehrenberg; planae lineae pelliculae cum materia organica; sine endobacteria; nucleus cum uno nucleolo._

**Diagnosis of Euglenidae classis nov. (ICZN); Euglenophyceidae subclassis nov. (ICBN)**

Phototrophic euglenids with one or two emergent flagella; osmotrophic euglenids with photosensory apparatus and/or vestigial plastids; ingestion apparatus, if present, of the MTR-pocket type. Composition: Suborder Euglenina Leedale 1967 and Suborder Eutreptiina Leedale 1967 emend. Busse et Preisfeld (ICZN); Order Euglenales Leedale 1967 and Order Eutreptiales Leedale 1967 emend. Busse et Preisfeld (ICBN).

**Latin diagnosis of Euglenophyceidae subclassis nov.**

_Euglenophyceae phototrophicae cum uno vel duobus flagellis emergentibus; Euglenophyceae osmotrophicae cum apparatu photosensorico et vel proplasto; apparatuse ingestionis typo ‘MTR-pocket’._


Phototrophic euglenids with two or more emergent flagella and flexible pellicle; eyespot present. Composition: _Eutreptia_, _Eutreptiella_ and _Tetreutreptia._

**Latin diagnosis of Eutreptiales Leedale 1967 emend. Busse et Preisfeld**

_Euglenophyceae phototrophicae cum duobus vel amplius flagellis emergentibus; cellularae metabolicae; cum stigma._

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