Mitochondrial and Plastid Genomes of the Monoraphid Diatom Schizostauron trachyderma

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Abstract: We provide for the first time the complete plastid and mitochondrial genomes of a monoraphid diatom: Schizostauron trachyderma. The mitogenome is 41,957 bp in size and displays two group II introns in the cox1 gene. The 187,029 bp plastid genome features the typical quadripartite architecture of diatom genomes. It contains a group II intron in the petB gene that overlaps the large single-copy and the inverted repeat region. There is also a group IB intron encoding a putative LAGLIDADG homing endonuclease in the rnl gene. The multigene phylogenies conducted provide more evidence of the proximity between S. trachyderma and fistula-bearing species of biraphid diatoms.

Keywords: monoraphid; biraphid; diatoms; organellar; genome; multigene; phylogeny; LAGLIDADG

1. Introduction

The genus Schizostauron Grunow [1] represents one of the heterovalvar lineages of diatoms, where the two primary shells (valves) which make up the siliceous cell wall (frustule) have differing morphologies. In the case of Schizostauron, only one valve possesses raphe—longitudinal slits at the center of the valve which are involved in motility. Diatoms with this particular kind of heterovalvity are called “monoraphids”, and the two valves are labelled by their raphe (RV) or lack of raphe (SV). Taxa in Schizostauron can be distinguished from other monoraphid genera by the morphology of the transverse thickening of silica at the central area of the RV called a stauros. The genus is typical of temperate to tropical marine littoral zones. Species in this genus have often been misidentified as the monoraphid genera Achnanthes or Cocconeis and have a complicated taxonomic history, which includes the registration of invalid holotypes [1–5].

Since the mid-19th century, monoraphid diatoms have been classified in a separate evolutionary lineage featuring one raphe-loss event [6–9]. Treated as such, all described monoraphid genera were assigned to the order Achnanthales, including Achnanthes (Achnanthaceae), Cocconeis, Campyloneis, Anorthoneis (Cocconeidaceae), Achnanthidium, and Eucocconeis (Achnanthidaceae). Round et al. [8] later split the genus Achnanthes sensu lato into several genera based on the re-evaluation of distinctive and shared morphological characters. The presence of a heterovalvate frustule is a shared feature of all members of Achnanthes sensu lato, but other morphological characters such as the girdle, orientation...
and shape of areola, and valve outline or central area structure are highly variable. The process of transferring taxa from *Achnanthes* and *Cocconeis* sensu lato into morphologically appropriate, newly established genera has continued over the last three decades and multiple new genera have been established [10–16], but despite these revisions, small groups of achnanthoid and cocconeoid taxa remain without detailed generic accommodation.

Molecular phylogenetic studies of the raphid diatoms have supported the idea of multiple switches to the monoraphid state. These studies have shown *Schizostauron* to be monophyletic and closely related to other monoraphid genera such as *Astartiella*, *Madinithidium*, *Kolbesia*, and *Karayevia*. However, these “stauroneid” monoraphid genera (so named because of the presence of *Stauroneis* Ehrenberg sensu stricto in the molecular clade) are not monophyletic with respect to the other monoraphid genera of the Achnanthaceae, Achnanthidiaceae, and Cocconeidaceae, suggesting that their monoraphid states evolved independently [4,5,17].

Among other discriminating morphological characters, *Schizostauron* and monoraphid species included in the stauroneid have coaxial internal proximal raphe ends. This character is universal for the stauroneid genera *Stauroneis*, *Craticula* [8], and others that were recently studied using molecular data such as *Fistulifera*, *Proschkinia*, and *Sternimirus* [18–21]. In contrast, the genera in the Achnanthidiaceae and Cocconeidaceae feature proximal raphe ends bent internally into opposite directions [8,22]. The monoraphid species clearly need a revision of the higher-level taxonomy as they fail to form a natural group [22–24]. In addition, this could bring us closer to understanding the evolutionary origins of the different monoraphid genera, which would be the first step to elucidating the ecological or molecular selective pressures which lead to heterovalv.

Here, we report the mitochondrial and plastid genome sequences of *Schizostauron trachyderma* (F.Meister) Górecka, Riaux-Gobin, and Witkowski, the first organellar genomes of a monoraphid diatom. These genomic data strengthen the phylogenetic position recently reported by Górecka et al. [5] and reveal that *Schizostauron* forms a clade within the Stauroneidaceae that is a sister to fistula-bearing *Fistulifera* and *Proschkinia* taxa. Several unusual features of the *S. trachyderma* organellar genomes will be discussed.

2. Results
2.1. Microscopy

Figure 1 illustrates the morphology of *S. trachyderma*, both in LM and SEM. The monoraphid morphology is apparent in comparing Figure 1d,e,i (RV) with Figure 1f–h (SV). The transverse stauros on the RV is particularly obvious in LM on Figure 1d,e.

2.2. Mitochondrial Genome

The 41,957 bp mitogenome of *S. trachyderma* (GenBank accession MZ520767) was retrieved with a coverage of 112X. It encodes the genes for 2 rRNAs, 22 tRNAs, and 35 proteins that include the two subunits of *Nad11* and the conserved orf147 [25] (Figure 2). Two group II introns of 3348 bp and 3325 bp in length interrupt *cox1* and both code for putative reverse transcriptases (orf692 and orf679, respectively). The *nad11-a* and *nad11-b* genes are adjacent to each other and are separated by only a 139-bp intergenic sequence. Note that the exact position of the start codon of *nad7* and *rpl6* remains ambiguous.
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2.3. Comparative Analysis of Diatom Mitochondrial Genomes

The mitochondrial genome of *S. trachyderma* was aligned to those of five closely related species identified in the phylogenetic analysis described below. In total, five blocks of synteny were detected in the MAUVE alignment (Figure 3). The mitogenome of *S. trachyderma* revealed a unique arrangement of these syntenic blocks, with the adjacent syntenic blocks formed by *cob* and by *cox3*, *nad3*, *cox2*, *nad7*, *nad9*, and *rps14* rearranged to the opposite DNA strand as compared to the other five genomes. Two sets of colinear mitochondrial genomes were identified: (1) those of Berkeleya fennica Juhlin-Dannfelt and Didymosphenia geminata (Lyngbye) M. Schmidt; and (2) those of Fistulifera solaris S. Mayama, M. Matsumoto, K. Nemoto, and T. Tanaka, as well as Fistulifera saprophila (Lange-Bertalot and Bonik) Lange-Bertalot and Proschkinia sp. These two sets of colinear mitogenomes differ only with respect to the position of a large syntenic block containing *atp6*, *rps10*, *rps8*, *rpl6*, *rps2*, *rps4*, *atp8*, *rps12*, *rps7*, *rpl14*, *rpl5*, *nad1*, *latC*, *orf147*, *rps11*, *rpl2*, *rps19*, *rps3*, *rpl16*, *atp9*, *nad4L*, *nad11-a*, and *nad11-b*. The significantly larger mitogenome of Proschkinia sp. is mostly explained by the presence of introns in *cox1*.
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2.4. Plastid Genome

The 187,029 bp plastid genome of *S. trachyderma* (GenBank accession MZ520768) exhibits the typical quadripartite architecture of diatom genomes (Figure 4). The large single-copy (LSC) region was retrieved with a coverage of 217X. It is 92,894 bp in size and encodes 67 conserved proteins, 15 tRNAs, and 22 open reading frames (ORFs) (Table 1). The closely linked orf104a and orf134a show similarities to *xerC* sequences coding for the putative integrases/recombinases. The putative protein of orf134a corresponds to the C-terminal domain of the integrases/recombinases and displays three of the conserved amino-acid residues present in this region (His-289, Arg-292, and Tyr-324), whereas the predicted protein of orf104a corresponds to the N-terminal domain of the integrases/recombinases but lacks the conserved Arg-173 typically present in this region. We speculate that these two ORFs are pseudogenes originating from a single large reading frame that was once coding for a functional protein. The predicted protein of orf110a shows some similarities with serine recombinases (*serC*), but its sequence is incomplete, lacking the C-terminal DNA binding site. The other putative *serC* (orf224a and orf227a) and *xerC* (orf299a and orf418a) proteins encoded by the plastid genome of *S. trachyderma* appear complete. In contrast, for example, to the *Haslea silbo* Gastineau, Hansen, and Mouget plastid genome that recently revealed all *serC* and *xerC* sequences in a single ca. 30 kb fragment located between *ycf35* and *psbA* [26], these elements are located in five distinct regions of the *S. trachyderma* (between the IR and *psaJ*, *ycf90* and *psbZ*, *psbB and rbcS*, *atpA* and *rps14*, *tsf* and *atpB*).
Figure 3. MAUVE alignment of the mitochondrial genomes of Berkeleya fennica (KM886611), Didymosphenia geminata (KX889125), Fistulifera saprophila (MT383640), Fistulifera solaris (KT363689), Proschkinia sp. (MH800316), and Schizostauron trachyderma (MZ520767). The legend below shows the gene content of the blocks of synteny (conserved protein-coding genes and rRNA only).

Table 1. List of the various ORF found in the mitochondrial and plastid genomes of Schizostauron trachyderma, with the name, position, putative conserved domains, and best blastp result.

| Name       | Position | Putative Conserved Domain | Best Blastp Result |
|------------|----------|---------------------------|--------------------|
| orf143a    | Plastid genome (LSC) | None                      | CAA45586.1 from Cylindrotheca closterium (2 × 10^{-58}, 69.23%) |
| orf227a    | Plastid genome (LSC) | Putative serine recombinase | AZJ16760.1 from Semiavis robusta (9 × 10^{-128}, 85.31%) |
| orf166a    | Plastid genome (LSC) | None                      | AZJ16664.1 from Semiavis robusta (2 × 10^{-24}, 36.88%) |
| orf123b    | Plastid genome (LSC) | None                      | WP_101018572.1 from Olleya sp. (3 × 10^{-6}, 34.29%) |
| orf148b    | Plastid genome (LSC) | None                      | AXF37983.1 from Semiavis robusta (6 × 10^{-35}, 66.41%) |
| orf406a    | Plastid genome (LSC) | None                      | CAA45586.1 from Cylindrotheca closterium (0.0, 91.75%) |
| orf152b    | Plastid genome (LSC) | None                      | YP_009028997.1 from Cylindrotheca closterium (2 × 10^{-33}, 44.30%) |
| orf418a    | Plastid genome (LSC) | Putative integrase recombinase | YP_009686230.1 from Halamphora calidilacuna (1 × 10^{-39}, 50.00%) |

Figure 4. Map of the plastid genome of S. trachyderma.
| Name       | Position       | Putative Conserved Domain       | Best Blastp Result (E-Value, Identity)                                                                 |
|------------|----------------|---------------------------------|--------------------------------------------------------------------------------------------------------|
| orf143a    | Plastid genome (LSC) | None                            | CAA45586.1 from Cylindrotheca closterium (2 × 10⁻⁵⁸, 69.23%)                                           |
| orf227a    | Plastid genome (LSC) | Putative serine recombinase      | AZJ16760.1 from Seminavis robusta (9 × 10⁻¹²⁸, 85.31%)                                                   |
| orf166a    | Plastid genome (LSC) | None                            | AZJ16664.1 from Seminavis robusta (2 × 10⁻²⁴, 36.88%)                                                   |
| orf123b    | Plastid genome (LSC) | None                            | WP_10118572.1 from Olleya sp. (3 × 10⁻⁶, 34.29%)                                                        |
| orf148b    | Plastid genome (LSC) | None                            | AXF37983.1 from Seminavis robusta (6 × 10⁻³⁵, 66.41%)                                                   |
| orf152b    | Plastid genome (LSC) | None                            | CAA45586.1 from Cylindrotheca closterium (0.0, 91.75%)                                                  |
| orf144a    | Plastid genome (SSC) | None                            | XP_009028997.1 from Cylindrotheca closterium (2 × 10⁻³⁷, 44.30%)                                        |
| orf190a    | Plastid genome (SSC) | None                            | XP_009686230.1 from Halaphora calidilacuna (1 × 10⁻⁵⁸, 50.00%)                                          |
| orf119     | Plastid genome (IR) | None                            | QUW40432.1 from Haslea silbo (5 × 10⁻²¹, 33.88%)                                                       |
| orf134a    | Plastid genome (LSC) | Putative integrase recombinase   | XP_009029005.1 from Cylindrotheca closterium (5 × 10⁻⁶³, 74.63%)                                       |
| orf104a    | Plastid genome (LSC) | Putative integrase recombinase   | XP_009686252.1 from Halaphora calidilacuna (2 × 10⁻²⁷, 56.31%)                                         |
| orf110a    | Plastid genome (LSC) | Putative serine recombinase      | HAC63963.1 from Cyanotheca sp. (1 × 10⁻¹¹⁹, 94.59%)                                                    |
| orf238b    | Plastid genome (LSC) | Putative serine recombinase      | QGW12742.1 from Nanofrustulum shiloi (1 × 10⁻⁴⁷, 85.26%)                                                |
| orf417b    | Plastid genome (LSC) | None                            | QUS63573.1 from Haslea silbo (3 × 10⁻²³, 35.02%)                                                       |
| orf224a    | Plastid genome (LSC) | Putative serine recombinase      | AXF37982.1 from Seminavis robusta (5 × 10⁻³³, 30.82%)                                                  |
| orf127a    | Plastid genome (LSC) | None                            | XP_009496149.1 from Plagiogrammopsis vanheurckii (6 × 10⁻¹³⁵, 88.79%)                                  |
| orf103a    | Plastid genome (LSC) | None                            | XP_009028999.1 from Cylindrotheca closterium (6 × 10⁻³⁷, 45.79%)                                       |
| orf158b    | Plastid genome (LSC) | None                            | XP_009029000.1 from Cylindrotheca closterium (4 × 10⁻⁵⁸, 52.94%)                                       |
| orf299a    | Plastid genome (LSC) | Putative integrase recombinase   | XP_009497021.1 from Psammones obaidii (7 × 10⁻¹⁵¹, 76.77%)                                              |
| orf305a    | Plastid genome (LSC) | None                            | AZJ16668.1 from Seminavis robusta (2 × 10⁻¹³⁴, 74.43%)                                                  |
| orf494a    | Plastid genome (IR) | Putative reverse transcriptase   | QGW12739.1 from Nanofrustulum shiloi (0.0, 58.17%)                                                     |
| orf119     | Plastid genome (IR) | Putative reverse transcriptase   | QGW12742.1 from Nanofrustulum shiloi (6 × 10⁻⁵⁶, 68.03%)                                                |
| orf139     | Plastid genome (IR) | Putative LAGLIDAG               | AAL34315.1 from Pterosperma cristatum (2 × 10⁻⁶⁶, 77.34%)                                               |
| orf100     | Plastid genome (IR) | None                            | AZJ16668.1 from Seminavis robusta (5 × 10⁻³², 68.29%)                                                  |
| orf190a    | Plastid genome (SSC) | None                            | XP_009308934.1 from Toxarium undulatum (2 × 10⁻⁴³, 61.96%)                                              |
| orf144a    | Plastid genome (SSC) | None                            | None significant                                                                                        |
| orf391a    | Plastid genome (SSC) | None                            | CAA45582.1 from Cylindrotheca fusiformis (2 × 10⁻¹⁶⁸, 73.63%)                                             |
| orf112a    | Plastid genome (SSC) | None                            | XP_009028833.1 from Asterionellopsis glacialis (1 × 10⁻⁴⁰, 90.00%)                                       |
| orf117c    | Plastid genome (SSC) | None                            | CAA45586.1 from Cylindrotheca closterium (2 × 10⁻⁵⁰, 74.77%)                                             |
The small single-copy (SSC) region of *S. trachyderma* was retrieved with a coverage of 222X. It is 59,661 bp in size, and encodes 52 conserved proteins, 7 tRNAs, and 16 non-conserved ORFs, some of which encode putative xerC (orf294a and orf317a) and serC (orf234a) integrases/recombinases. The predicted protein of orf112a also shows some similarities with xerC integrases/recombinases but only retains the conserved Arg-173 and lacks the C-terminal region. Note that most of the ORFs encoding putative integrases/recombinases are located in a single block between *ycf46* and *rps10*.  

The 17,237 bp inverted repeat (IR) region of the *S. trachyderma* plastid genome was retrieved with a coverage of 428X. It encodes nine proteins (*psbY*, *ycf89*, *ycf45*, *rpl20*, *rpl35*, *psaE*, *ftsH*, *petD*, *petB*), three rRNAs, three tRNAs, and three ORFs. The *petB* gene overlaps the IR and LSC regions and contains a 2287-bp group II intron coding for a putative reverse transcriptase (RT, orf494) that shows a high sequence similarity to the RTs identified in the same gene from the diatoms *Nanofrustulum shiloi* (J.J. Lee, Reimer, and McEnery) Round, Hallsteinsen and Paasche [27] and *Halamphora calidilacuna* J.G. Stepanek and Kociolek [28] (Table 1). Moreover, the introns of *S. trachyderma*, *N. shiloi*, and *H. calidilacuna* share the same insertion position between codons 7 and 8 of *petB*. Located between *ftsH* and *petB* of *S. trachyderma*, orf119 shows some similarities to serC sequences, but its N-terminal region is incomplete and lacks most of the presynaptic site 1 dimer interface.  

The plastid gene for the large subunit ribosomal RNA (*rnl*) of *S. trachyderma* contains an IB4 intron of 657 bp that encodes a putative LAGLIDADG homing endonuclease (orf139). This intron is inserted between residues 1931 and 1932 relative to the 23S rRNA of *Escherichia coli* str. K-12, an insertion site that has been frequently observed among green algae [29,30]. The predicted protein of orf139 also shows strong similarity with the homing endonucleases identified in green algal IB4 introns at site 1931 [29]. As shown in the LOGO consensus of Figure 5, the typical QWIVGFVDG and PFFE motifs of LAGLIDADG homing endonucleases are highly conserved in the predicted protein of orf139.

### Table 1. Cont.

| Name     | Position        | Putative Conserved Domain                          | Best Blastp Result (E-Value, Identity) |
|----------|-----------------|---------------------------------------------------|----------------------------------------|
| orf152a  | Plastid genome (SSC) | None                                               | HAC63961.1 from *Cyanothece* sp. (3 × 10^{-102}, 97.32%) |
| orf317a  | Plastid genome (SSC) | Putative integrase recombinase                      | NNF85095.1 from *Winogradskyella* sp. (4 × 10^{-88}, 53.01%) |
| orf290b  | Plastid genome (SSC) | None                                               | YP_00928832.1 from *Asterionellopsis glacialis* (8 × 10^{-61}, 51.33%) |
| orf188a  | Plastid genome (SSC) | None                                               | AZJ16664.1 from *Seminavis robusta* (3 × 10^{-93}, 76.63%) |
| orf234a  | Plastid genome (SSC) | Putative serine recombinase                         | QWM93463.1 from *Tryblionella apiculata* (1 × 10^{-140}, 88.53%) |
| orf148a  | Plastid genome (SSC) | None                                               | YP_009059189.1 from *Eunotia naegelii* (3 × 10^{-15}, 32.89%) |
| orf313a  | Plastid genome (SSC) | None                                               | None significant                       |
| orf294a  | Plastid genome (SSC) | Putative integrase recombinase                      | YP_009495909.1 from *Plagiogramma staurophorum* (6 × 10^{-176}, 83.33%) |
| orf516a  | Plastid genome (SSC) | None                                               | CAA45586.1 from *Cylindrotheca closterium* (2 × 10^{-92}, 34.58%) |
| orf645   | Mitogenome       | Putative reverse transcriptase                      | YP_009317775.1 from *Navicula ramosissima* (0.0, 82.20%) |
| orf690   | Mitogenome       | Putative reverse transcriptase                      | QUS63794.1 from *Haslea silbo* (0.0, 93.14%) |

2.5. Comparative Analyses of the Gene Content of Diatom Plastid Genomes

The gene contents of plastid genomes from all available raphid diatoms were compared with that of *S. trachyderma*; a comparative table adapted from previous work [31] is displayed as Figure 6. The LSC contains a copy of the *tsf* gene, which codes for translation elongation factor T and often seems lost among raphid pennates, found only in
Fistulifera spp., D. geminata, Gomphoneis minuta var. cassiae Kociolek and Stoermer, and Phaeodactylum tricornutum Bohlin. With the exception of P. tricornutum, all these species belong to the same clade in the plastid multigene phylogeny presented below, but this clade also contains Climaconeis spp., among which this gene was not found [32]. None of the Bacillariaceae (Nitzschia spp., Tryblionella apiculata W.Gregory) or Naviculaceae (Haslea spp., Seminavis robusta D.B. Danielidis and D.G.Mann, Navicula veneta Kützing) present it, nor do Halamphora spp. However, based on previous works [31], this gene is also absent from almost three-quarters of the plastid genomes sequenced, including most centric and araphid pennate species. Schizostauron trachyderma possesses the two genes thiS and thiG, shared by all the other species of the cluster, except Fistulifera spp. Schizostauron trachyderma, which is missing the bas1/ycf42 gene. This gene is also missing among most Bacillariaceae and Naviculaceae but has been found in Nitzschia supralitorea Lange-Bertalot. Among the species clustered by phylogeny, only D. geminata presents a pseudogene version of bas1/ycf42.

Figure 5. Sequence motifs obtained by aligning LAGLIDADG proteins from Schizostauron trachyderma and 13 sequences from various species of Chlorophyceae corresponding to IB4-L8 LAGLIDADG proteins. The alignment displayed as a LOGO shows the presence of two conserved motifs, QWIVGFVDG and PFFE.
Figure 6. Comparison of the gene composition for the plastid genomes of all the species used in the phylogeny below. A 1/blue indicates the presence of the gene, a 0/white its lack, and a Ψ/green a pseudogene version of the gene.
2.6. Multigene Phylogenies

We inferred mitochondrial and plastid phylogenomic trees from the concatenated gene sequences of *S. trachyderma* and all raphid pennate diatom organelle genome sequences available in GenBank, using an araphid pennate species as an outgroup. *Schizostauron trachyderma* proved to be a sister to *Fistulifera* spp. (and *Proschkinia* sp. in the case of the mitochondrial data) in all trees (Figures 7 and 8), a result consistent with recently reported phylogenetic analyses [5,20,21]. Note that *Fistulifera* and *Proschkinia* taxa present a distinctive occluded pore on their valves, called a fistula. Interestingly, this feature is not found on *Schizostauron* valves.

The mitogenome-based tree (Figure 7) is the most taxon-rich of both trees, mostly because of the availability of several mitogenomes from two genera of the Bacillariaceae (*Nitzschia* and *Pseudo-nitzschia*). In addition to the *Schizostauron, Fistulifera*, and *Proschkinia* association mentioned above, the mitochondrial tree revealed that *S. trachyderma* was part of a larger clade including *Surirella* sp., *Halamphora* spp., *Entomoneis* sp., *P. tricornutum*, *D. geminata*, and *B. fennica*. The Naviculaceae formed a monophyletic group sister to a clade containing the rest of the raphid pennates.

**Figure 7.** Maximum likelihood phylogeny inferred from an alignment of concatenated protein-coding genes from 37 mitochondrial genomes of diatoms, including that of *S. trachyderma*. The araphid species *Ulnaria acus* served as an outgroup in this analysis. The best scoring RAxML tree (log likelihood = $-414,469.369802$) is presented with bootstrap support values denoted on the nodes. The scale bar indicates the number of substitutions per site. The araphid species *Ulnaria acus* is used as an outgroup.
The best scoring RAxML tree (log likelihood = -878,702.878358) is presented with bootstrap support values denoted on the nodes. The scale bar indicates the number of substitutions per site.

The mitogenome-based tree (Figure 7) is the most taxon-rich of both trees, mostly because of the availability of several mitogenomes from two genera of the Bacillariaceae (Nitzschia and Pseudo-nitzschia). In addition to the Schizostauron, Fistulifera, and Proschkinia association mentioned above, the mitochondrial tree revealed that S. trachyderma was part of a larger clade including Surirella sp., Halamphora spp., Entomoneis sp., P. tricornutum, D. geminata, and B. fennica. The Naviculaceae formed a monophyletic group sister to a clade containing the rest of the raphid pinnate.

Although the plastome-based phylogenetic tree (Figure 8) has a smaller taxon sampling, its topology is similar to the mitochondrial tree. The S. trachyderma also proved to be a sister of Fistulifera spp. and these taxa were recovered in a clade that also includes G. minuta var. cassiae, D. geminata, and Climaconis spp.

3. Discussion

The multigene phylogenetic analyses presented here confirm the close evolutionary relationship between S. trachyderma and fistula-bearing taxa such as Fistulifera spp. and Proschkinia sp. This phylogenetic association could be improved and refined in future studies of additional organellar genomes of monoraphid genera such as Astartillia, Maiadinithidium, Karayevia, and Kolbesia. Based on recently published analyses, we expect these genera to be related to Schizostauron, as these stauroneid monoraphid taxa form a monophyletic clade sister to Fistulifera and Proschkinia, which is nested in a clade comprising genera belonging to Stauroneidiaceae, such as Stauroneis, Criculula, Sternimirus, Dorofeyukia, Parlibellus, and Prestauroneis [5,17,18,20,21]. It is possible that the close relationship of stauroneid biraphid genera to stauroneid monoraphid genera is a result of insufficient taxon sampling or inadequate or insufficient choice of molecular markers. Genomic data from these taxa might reveal signature characters of specific monoraphid clades and could provide insights to the independent losses of the raphe on one valve.

The S. trachyderma plastid genome revealed features that were rarely or never observed among diatoms. The finding of a group II intron overlapping the IR and the LSC regions in the petB gene is certainly noteworthy, as is the observation of a LAGLIDADG homing endonuclease in the rnl gene. To our knowledge, genes encoding this type of homing

Figure 8. Maximum likelihood phylogeny inferred from an alignment of concatenated protein-coding genes from 26 plastid genomes of diatoms, including that of S. trachyderma. The araphid species Ularia acus served as an outgroup in this analysis. The best scoring RAxML tree (log likelihood = -878,702.878358) is presented with bootstrap support values denoted on the nodes. The scale bar indicates the number of substitutions per site.
endonuclease have so far been found only in the IA3 rnl intron of the S. robusta plastid genome (annotated as I-SroI, accession AZJ16657.1 [33] and in a cox1 intron of the N. supralitorea mitogenome, accession QWM93242.1 [34]. As in a few other diatom species [31], our analyses of conserved domains in the putative serC and xerC genes of S. trachyderma suggest that some of them are pseudogenes. As interesting as both the above-mentioned results are for the study of mobile DNA, we refrain from speculating on any explanation regarding their presence/absence among different taxa.

Our study, the first of its kind on a monoraphid diatom, should soon be followed by more organellar genome sequencing on other species belonging to genera such as the aforementioned Parlibellus, Stauroneis, Astartiella, and Madinithidium.

4. Materials and Methods

4.1. Isolation and Cultivation of the Biological Material

The strain SZCZE1420 of S. trachyderma was isolated from an environmental sample collected in February 2015 near Jeddah on the Red Sea coast of Saudi Arabia (21.7561° N 39.05° E). A monoclonal culture was established using glass micropipettes and inverted light microscopy (Nikon eclipse TS100) following Andersen and Kawachi [35]. The culture is kept in artificial f/2 culture medium [36] in a growth chamber (Biogenet, Poland) with 12 h day (20 °C):12 h night (18 °C) cycles under 100 µmol photons m⁻² s⁻¹ illumination.

4.2. Light and Scanning Electron Microscopy

Diatom pellets were centrifuged at 900 × g for 5 min and treated with 37% hydrogen peroxide for 3 h at 170 °C to remove organic components of frustules. The residual material was washed 7–10 times with distilled water. For light microscopy (LM), cleaned frustules were pipetted onto coverslips, air-dried, and mounted on glass slides with synthetic diatom resin Naphrax® (Brunel Microscopes Ltd., Chippenham, UK). LM microphotographs of cleaned frustules and plastids were taken at the University of Szczecin by means of a Zeiss Axio Scope A1 (Carl Zeiss, Jena, Germany) with an oil immersion lens Zeiss Plan-Apochromat 100×/1.40 Oil M27 (Carl Zeiss, Jena, Germany) using a Canon EOS 500D camera (Canon, Tokyo, Japan) with the Canon EOS Utility software. For scanning electron microscopy (SEM), the cleaned material was pipetted onto a Whatman Nuclepore polycarbonate membrane filter with 5 µm pores (cat. no. 110613, Maidstone, UK) and mounted onto aluminium stubs. SEM observations were performed using a Hitachi SU8010 (Tokyo, Japan) at the University of Rzeszów (Poland), Faculty of Agriculture and Biology.

4.3. DNA Sequencing, Annotation, Whole Genome Alignments and Phylogeny

The SZCZE1420 clone of S. trachyderma was grown as described above, and cells in the exponential growth phase were harvested by centrifugation. DNA was extracted according to Doyle and Doyle [37]. Sequencing took place on the DNBseq platform at the Beijing Genomics Institute (Shenzhen). A total of 40 million 150-bp paired-end reads were assembled using SPAdes 3.14.0 [38] with a k-mer of 125. Contigs were verified and merged using Consed [39]. The genes were identified as previously described [34,40]. Maps of organellar genomes were prepared using the OGDRAW v1.3.1 online platform [41]. Whole mitogenome alignment was performed with progressive Mauve [42] after removing the second copy of the IR sequence in the plastid genomes. LOGO consensus sequences of LAGLIDADG homing endonucleases were obtained online with WebLogo3 [43]. For multigene phylogenies, mitochondrial and plastid protein-coding genes were extracted, separately concatenated, and aligned with orthologous diatom gene sequences obtained from GenBank. The mitochondrial and plastid multigene phylogeny were conducted on 37 and 26 taxa, respectively, including S. trachyderma and U. acus in both cases. The sampling was restricted to raphid pennate species, except for the araphid species Ulnaria acus (Kützing) Aboal that served as an outgroup. Phylogenetic analyses were conducted using RaxML version 8 [44], with the GTR + I + G model and 1000 bootstrap replications. Genes were concatenated and aligned using MAFFT 7 [45] before the alignments
were trimmed by trimAl v1.2 [46]. The evolution model was chosen using jModelTest2 v2.1.10 [47] on the trimmed alignments. The number of concatenated genes was 35 for the mitochondrial-inferred phylogeny and 127 for the plastid-inferred phylogeny. The final sizes of the alignments as calculated by trimAl were 22140 bp for the mitochondrial genes alignment and 86587 bp for the plastid genes alignment. The evolution model was used as a single model across the entire alignment.

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