

Article



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Molecular and morphological investigation of *Sellaphora ashinovii sp. nov.* and *Planothidium paisiusii sp. nov.*—two new diatom species from Oromia Region, Ethiopia

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Abstract

Two diatom species from Ethiopian freshwater ecosystems, *Sellaphora ashinovii sp. nov.* and *Planothidium paisiusii sp. nov.*, are described as new to science. Their morphology and molecular characteristics were analysed to establish their taxonomic position. Comparisons with closely related taxa and results of molecular analyses are presented. This study adds to the limited molecular information available on African diatoms and highlights the need for further molecular surveys across the continent.

Key words: diatoms, Ethiopia, molecular analysis, new species, Planothidium, Sellaphora

Introduction

Diatoms of Africa have been attracting the attention of researchers for at least 180 years, beginning with the work of Ehrenberg (1845). In the 20th century, researchers such as O.F. Müller, F. Hustedt, B.J. Cholnoky, N. Foged, F.R. Schoeman, R.E.M. Archibald, and others did extensive work on the diatom flora of the continent. The study of diatom biodiversity continues nowadays as well, with new genera and species being described (Cocquyt & Ryken 2016; Rybak *et al.* 2021; Taylor *et al.* 2014, 2024, etc.). These works were predominantly focused on Central and South Africa, while East Africa, and Ethiopia in particular, is much less well studied in this regard. Most of the studies in Ethiopia were focused on the fossil diatoms of the Afar Depression, Ethiopian Rift Valley (Gasse 1974a,b; Gasse & Tekaïa 1979; Telford & Lamb 1999; Roubeix *et al.* 2014; Mohan *et al.* 2016; Grady *et al.* 2020; Hunegnaw & Getaneh 2024).

The study of modern diatoms in this region began only lately. Works have been published concerning the species composition of phytoplankton (Kebede & Belay 1994), biomonitoring in rivers, the structure of algal communities (Beyene & Triest 2009; Wondmagegn *et al.* 2019; Shibabaw *et al.* 2021), as well as descriptions of new diatom species discovered from fossil sediments (Mohan *et al.* 2016; Grady *et al.* 2020). The first strains of Ethiopian diatoms isolated from Abaya Lake were used in the work of Kulikovskiy *et al.* (2020); they were identified as *Gogorevia uniseriata* (Yan Shi & B.-H. Kim) Kulikovskiy & Kociolek in Kulikovskiy *et al.* (2020: 1607), analyzed morphologically, and afterwards used in phylogenetic studies. Despite the rich aquatic ecosystems of Ethiopia, no new diatom species have yet been described from the region using molecular approaches.

The genus *Sellaphora* Mereschkowsky (1902: 186) was first described in 1902 with the type species *Sellaphora pupula* (Kützing) Mereschkowsky (1902: 187) and currently includes about 250 species (Guiry & Guiry 2025).

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Sellaphora has been widely studied, covering valve morphology, chloroplast structure and sexual reproduction (Mann 1989; Mann et al. 1999, 2004; Mann & Stickle 2009; Vanormelingen et al. 2013, etc.). Molecular methods have demonstrated that the genus is monophyletic (Evans et al. 2007, 2008, 2009), however, morphologically it is quite diverse and has been divided into several groups by different authors based on morphology (Mann et al. 2008; Liu et al. 2020). Recent taxonomic investigations of Sellaphora include revisions of complicated species groups (Mann et al. 2004, 2008; Wetzel et al. 2015), as well as descriptions of new species from various geographical locations (Andreeva et al. 2018; Liu et al. 2020; Kochoska et al. 2021; Glushchenko et al. 2022, 2023; Solak et al. 2023 and others).

Planothidium Round & Bukhtiyarova (1996: 351) was established by Round and Bukhtiyarova (1996) with Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot (1999: 287) as the type species. The genus currently includes more than 120 species (Guiry & Guiry 2025) that are divided into several morphological groups based on the structure of the rapheless valve (Morales 2006). Molecular investigations of Planothidium have shown that these morphological groups form separate clades on the phylogenetic tree (Jahn et al. 2017) and demonstrated the presence of cryptic diversity in species complexes such as P. lanceolatum, Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot (1999: 282) and Planothidium victori Novis, Braidwood & Kilroy in Novis et al. (2012: 22) (Tseplik et al. 2024a, b). In recent years, taxonomic studies of Planothidium focused on species from all morphological groups and included reinvestigations of type materials of such species as P. lanceolatum (Van de Vijver et al. 2013), P. frequentissimum, Planothidium rostratum (Østrup) Lange-Bertalot (1999: 279) (Wetzel et al. 2019), Planothidium delicatulum (Kützing) Round & Bukhtiyarova (1996: 353) (Van de Vijver et al. 2018), as well as descriptions of new species based on both morphological and molecular data (Jahn et al. 2017; Van de Vijver et al. 2018; Stancheva 2019; Wetzel et al. 2019; Juchem et al. 2023; Junqueira et al. 2024, etc.).

Although *Sellaphora* and *Planothidium* have been extensively studied across Europe, Asia, and the Americas, investigations from African freshwater ecosystems remain rare. To date, no new species of these genera have been described from Ethiopia, particularly using an integrative approach combining morphology and molecular methods. Given the ecological importance and hidden diversity revealed by recent molecular studies in other regions, there is a strong need to document and characterise African representatives. In this study, we describe one new species each of *Sellaphora* and *Planothidium* from Ethiopian freshwater habitats, supported by detailed morphological analysis and molecular evidence.

Materials and methods

Sampling. Samples from Ethiopia were collected by B.A. Levin (IBIW RAS) in April 2016 under the umbrella of the Joint Ethiopian-Russian Biological Expedition (JERBE). The details of samples are given in Table 1.

TABLE 1. List of strains used in this study.

Strain	Sampling locality	Collection date	Sample type	Coordinates	Elevation	Sample no.
Sellaphora ashinovii sp. nov. CBMC129ef	Oromia Region, Lake Langano	15 April 2016	periphyton	N 7°38.85' E 38°49.176'	1591 m	E67
Planothidium paisiusii sp. nov. CBMC155ef	Mesekelhericho, Adaba, Oromia Region, West Arsi, nameless mountain stream	3 April 2016	periphyton	N 07°1.96' E 39°33.277'	2978 m	E14

Culturing. Part of each sample was transferred to the WC liquid culturing medium (Guillard & Lorenzen 1972). Monoclonal strains were established by micropipetting single cells under an inverted microscope Axio Vert. A1 (Zeiss, Oberkochen, Germany). Non-axenic unialgal cultures were maintained in the WC culturing medium at room temperature 22–25°C in a growth chamber illuminated by white diodes with a light intensity of 100 lx with a 12:12 h light:dark photoperiod for approximately 1 month before performing microscopical investigation and DNA extraction. The strains were deposited in the Culture and Barcode Collection of Microalgae and Cyanobacteria "Algabank" (CBMC) at K.A. Timiryazev Institute of Plant Physiology RAS.

Preparation of slides and microscope investigation. Cells for LM and SEM investigations were processed with a standard procedure that involves boiling the material in concentrated hydrogen peroxide (≈37%) in order to remove organic matter. Distilled water was used to wash the material. Permanent diatom preparations were mounted

in Naphrax® mounting medium (Naphrax®, Brunel Microscopes Ltd., Chippenham, UK). Light microscopic (LM) observations were made using the microscope AxioScope A1 (Zeiss, Oberkochen, Germany) with an oil immersion objective (×100/n.a.1.4, DIC) and an Axiocam Erc 5s camera (Zeiss, Oberkochen, Germany). For scanning electron microscopy (SEM), parts of the suspensions were fixed on aluminium stubs after air-drying. The stubs were sputter-coated with 50 nm Au in an Eiko IB-3 (Eiko Engineering, Ltd., Hitachinaka, Japan). Ultrastructure of the valves was examined with the scanning electron microscope JSM-6510LV (Jeol, Tokyo, Japan). Acquired images were processed using the Adobe Photoshop software.

Molecular study. Genomic DNA of the studied diatom strains was extracted from fresh cultures by Chelex 100 Chelating Resin (Bio-Rad Laboratories, Hercules, CA, USA) using protocol 2.2. Nuclear gene 18S rRNA and plastid *rbc*L gene were amplified. For the highly variable V4 region of 18S rDNA (393–403 bp) D512for and D978rev primers were used (Zimmermann *et al.* 2011). The plastid *rbc*L (636–708 bp) was amplified using rbcL404+ and rbcL1444-(Ruck & Theriot 2011) primers (see Table 2 for full primer sequences).

TABLE 2. Primers used for molecular study.

Primer	Primer sequence	Source
D512for	ATT CCA GCT CCA ATA GCG	Zimmermann et al. 2011
D978rev	GAC TAC GAT GGT ATC TAA TC	Zimmermann et al. 2011
rbcL404+	GCT TTA CGT TTA GAA GAT ATG	Ruck & Theriot 2011
rbcL1444-	GCG AAA TCA GCT GTA TCT GTW G	Ruck & Theriot 2011

PCR amplifications were performed using premade mastermixes (ScreenMix, Evrogen, Moscow, Russia) (containing Taq polymerase and 2 mM Mg²⁺). The amplification of the 18S rDNA was performed using the following program: 5 min of denaturation at 95 °C; followed by 35 cycles of denaturation at 94 °C (30 s), annealing at 52 °C (30 s), and elongation at 72 °C (50 s); with a final extension at 72 °C (7 min), subsequently held at 12 °C. The amplification of the *rbc*L gene was performed using the following program: 4 min of denaturation at 94 °C; followed by 44 cycles of denaturation at 94 °C (50 s), annealing at 53 °C (50 s), and elongation at 72 °C (80 s); with a final extension at 72 °C (10 min), subsequently held at 12 °C.

The PCR products were visualized on a 1.0% agarose gel stained with SYBR™ Safe (Life Technologies, Carlsbad, CA, USA) and then purified using a mixture of FastAP, 10× FastAP Buffer, Exonuclease I (Thermo Fisher Scientific, Waltham, MA, USA), and water. The purified PCR products were sequenced by Sanger Sequencing method using a Genetic Analyzer 3500 instrument (Applied Biosystems, Waltham, MA, USA) with the same primers as for the PCR amplification.

Newly obtained sequences were manually edited in Ridom TraceEdit ver. 1.1.0 (Ridom GmbH, Münster, Germany) and Mega ver. 7 software (Kumar et al. 2016). For the phylogeny reconstruction for the strain of the genus Planothidium, the reads of the CBMC155ef strain were included in the alignments with sequences of 53 diatom species downloaded from GenBank (taxa names and accession numbers are given in Figure 64). Diatom species from genus Cocconeis were chosen as the outgroup. The reads of Sellaphora strain CBMC129ef were included in the alignments along with corresponding sequences of 43 diatom species downloaded from GenBank (taxa names and accession numbers are given in Figure 26). Centric diatom species from genera Stephanodiscus and Skeletonema were chosen as the outgroups. The nucleotide sequences of the 18S rRNA and rbcL genes were aligned separately using the Mafft ver. 7 software and the E-INS-i model (Katoh & Toh 2010). The final alignments were then carried out: unpaired sites were visually determined and removed from the beginning and the end of the resulting matrices. For the protein-coding sequences of the rbcL gene, we checked that the beginning of the aligned matrix corresponds to the first position of the codon (triplet). The resulting alignments had lengths of 404 (18S rDNA for Planothidium strains), 409 (18S rDNA for Sellaphora strains), 636 (rbcL for Planothidium strains), and 708 (rbcL for Sellaphora strains) characters. After removal of the unpaired regions, the aligned 18S rRNA gene sequences were combined with the rbcL gene into a single matrix using Mega ver. 7 software.

The Bayesian inference (BI) method was performed to infer the phylogenetic position of new diatom strains using Beast ver. 1.10.1 software (BEAST Developers, Auckland, New Zealand) (Drummond, Rambaut, 2007). The most appropriate partition-specific substitution models, shape parameter α and a proportion of invariable sites (pinvar) were recognized by the Bayesian information criterion (BIC) in jModel-Test ver. 2.1.10 software (Vigo, Spain) (Darriba *et al.* 2012). This BIC-based model selection procedure selected the following models, shape parameter α and a proportion of invariable sites (pinvar) for the *Planothidium* tree: TrN+I+G, α =0.3290 and pinvar=0.6120 for 18S rDNA; TPM1uf

+I, pinvar=0.8040 for the first codon position of the rbcL gene; JC+I, pinvar= 0.7800 for the second codon position of the rbcL gene; HKY+G, α =0.6710 for the third codon position of the rbcL gene. This BIC-based model selection procedure selected the following models, shape parameter α and a pinvar for the *Sellaphora* tree: TrN+I+G, α =0.4930 and pinvar=0.6400 for 18S rDNA; HKY+I+G, α =0.3740 and pinvar=0.6580 for the first codon position of the rbcL gene; JC+I and pinvar=0.8940 for the second codon position of the rbcL gene; HKY+G and α =0.3980 for the third codon position of the rbcL gene. However, the HKY model was applied instead of TrN, TPM1uf, and the GTR applied instead of JC, TrN as the most similar applicable options for BI. A speciation model was performed by a Yule process tree prior. Five MCMC analyses were run for 7 million generations (burn-in 1,000 million generations). The convergence diagnostics was performed in the Tracer ver. 1.7.1 software (MCMC Trace Analysis Tool, Edinburgh, United Kingdom) (Drummond & Rambaut 2007). The initial 15% trees were removed, the rest retained to construct a final chronogram with 90% posterior probabilities. The robustness of tree topologies was assessed by boot-strapping the data set with Maximum Likelihood (ML) analysis using RAxML software (Stamatakis *et al.* 2008). The ML bootstrapping was performed with 1,000 replicas. Trees were viewed and edited using FigTree ver. 1.4.4 (University of Edinburgh, Edinburgh, United Kingdom) and Adobe Photoshop CC ver. 19.0 software.

Results

Sellaphora ashinovii Glushchenko, Tseplik, Maltsev, Genkal, Iurmanov & Kulikovskiy sp. nov. (Figs 1-25)

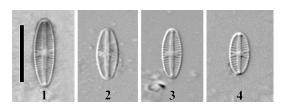
Holotype. Slide no. 05556 (represented here by Figure 13), deposited in the Herbarium of K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences (HD), Moscow, Russia, prepared from oxidized culture strain CBMC129ef.

Isotype. Slide no. 05556a, Herbarium of Addis Ababa University, Addis Ababa, Ethiopia.

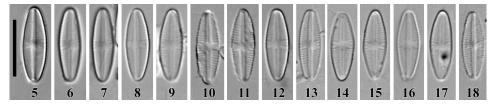
Reference strain. CBMC129ef from the Culture and Barcode Collection of Microalgae and Cyanobacteria "Algabank" (CBMC), isolated from sample E67.

Type locality. Ethiopia, Oromia Region, Lake Langano, periphyton, 1591 m elev., 7°38.85'N 38°49.176'E, *leg.* B.A. Levin, 15 April 2016. Sample E67.

Description. LM (Figs 1–18). Valves linear-lanceolate with rounded ends. Length 7.4–13.5 μm (11.9 \pm 1.6; n=30), width 3.5–4.5 μm (4.0 \pm 0.3; n=30). Axial area narrow, weakly expanded towards the central area. Central area bowtie-shaped, formed by 3–4 shortened striae on each side. Raphe filiform. Striae weakly radiate, 24–26 in 10 μm.

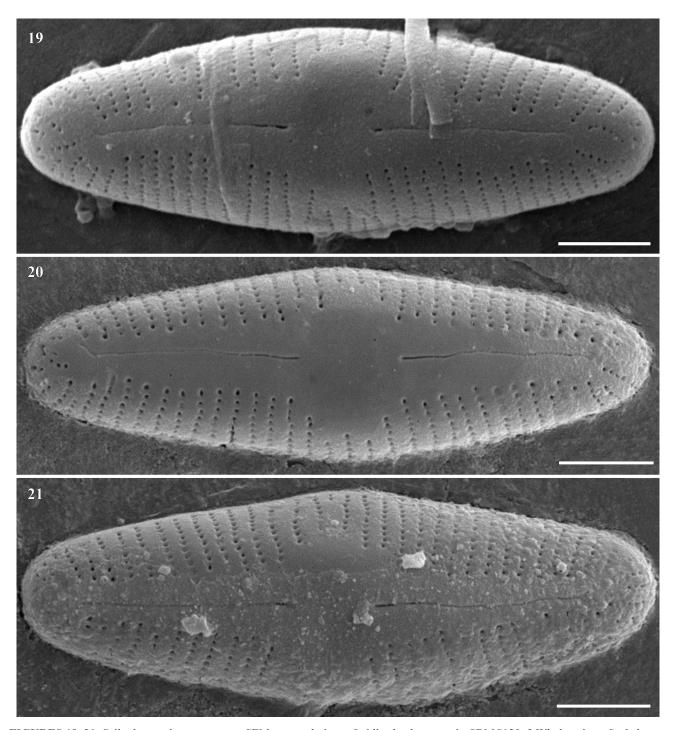


FIGURES 1-4. Sellaphora ashinovii sp. nov. LM, DIC. Sample of wild population, size diminution series. Scale bar = 10 µm.



FIGURES 5–18. Sellaphora ashinovii sp. nov. LM, DIC. Slide no. 05556 from oxidized culture, strain CBMC129ef. Holotype represented by fig. 13. Scale bar = $10 \mu m$.

SEM. External views (Figs 19–21). Valve face slightly convex. Raphe weakly lateral. Central raphe ends slightly expanded. In the initial valves, distal raphe ends terminate on valve face. One of the distal raphe ends is curved, the other is almost straight. Striae uniseriate. Shortened intercalary striae and short striae on the valve apices are present. Areolae small, rounded, ca. 70 in 10 µm.



FIGURES 19–21. *Sellaphora ashinovii sp. nov.* SEM, external views. Oxidized culture, strain CBMC129ef. Whole valves. Scale bars = 2 μm.

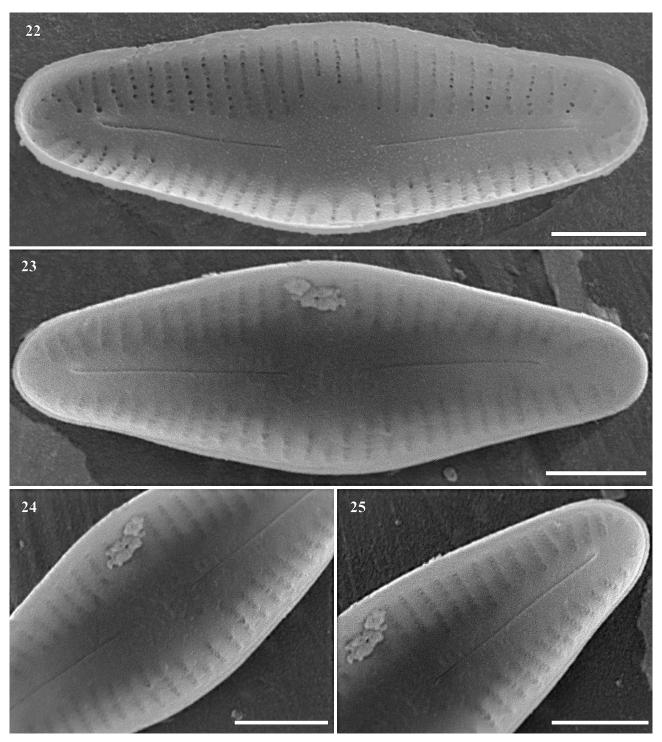
SEM. Internal views (Figs 22–25). Raphe branches almost straight, filiform. Central raphe ends weakly unilaterally deflected. Distal raphe ends terminating in small helictoglossae. Shortened intercalary striae present. Areolae covered by individual hymenes.

Sequence data. Partial 18S rDNA gene sequence comprising V4 domain sequence (GenBank accession number PV383267) and partial *rbc*L sequence (GenBank accession number PV384976) for the strain CBMC129ef.

Etymology. This species is named after Nikolay Ivanovich Ashinov (1859–1902), a renowned Russian Cossack chieftain and traveler in Africa, who made significant contributions to the development of Russia-Abyssinia relations. He was also an amateur linguist who published the book "The Abyssinian alphabet and the initial Abyssinian-Russian dictionary".

Distribution. As yet known only from the type locality.

Molecular study. *S. ashinovii sp. nov.* forms an independent branch on the phylogenetic tree (Fig. 26) that is sister to strains of *Sellaphora minima* (Grunow) D.G. Mann in Round *et al.* (1990: 552). These strains are part of a larger clade that also includes *Sellaphora seminulum* (Grunow) D.G. Mann (1989: 2) and *S. terrestris* Glushchenko, Kezlya, Maltsev & Kulikovskiy in Glushchenko *et al.* (2022: 2).



FIGURES 22–25. *Sellaphora ashinovii sp. nov.* SEM, internal views. Oxidized culture, strain CBMC129ef. Figs 22, 23. Whole valves. Fig. 24. Central area. Fig. 25. Valve end. Scale bars = 2 µm.

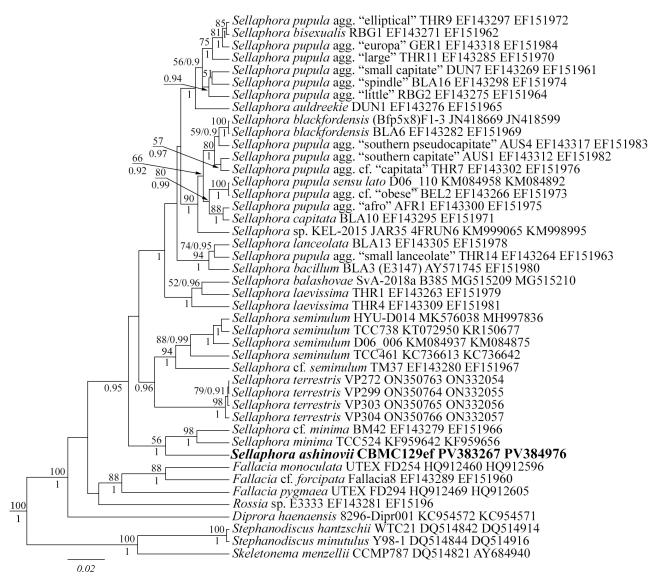


FIGURE 26. Phylogenetic position of *Sellaphora ashinovii sp. nov.* (indicated in bold) within the Sellaphoraceae based on Bayesian inference for a concatenated alignment of 44 partial *rbcL* and partial 18S rDNA sequences. The total length of the alignment is 1,117 characters. Values above the horizontal lines are bootstrap support from ML analyses (< 50 are not shown); values below the horizontal lines (or to the right of the slash) are Bayesian posterior probabilities (< 0.9 are not shown). Strain numbers (if available) and GenBank numbers are indicated for all sequences.

Planothidium paisiusii Tseplik, Glushchenko, Maltsev, Genkal, Iurmanov & Kulikovskiy sp. nov. (Figs 27-63)

Holotype. Slide no. 05583 (represented here by Figure 49), deposited in the Herbarium of K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences (HD), Moscow, Russia, prepared from oxidized culture strain CBMC155ef.

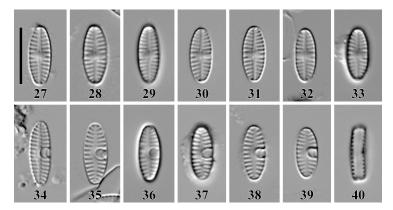
Isotype. Slide no. 05583a, Herbarium of Addis Ababa University, Addis Ababa, Ethiopia.

Reference strain. CBMC155ef from the Culture and Barcode Collection of Microalgae and Cyanobacteria "Algabank" (CBMC), isolated from sample E14.

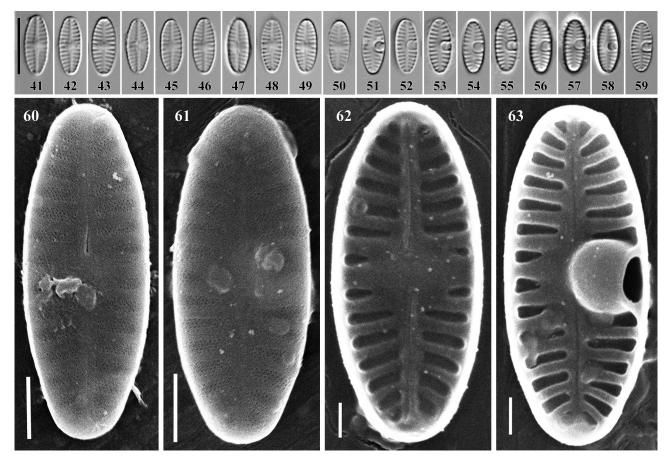
Type locality. Ethiopia, Mesekelhericho, Adaba, West Arsi, nameless mountain stream, periphyton, 2978 m elev., 07°1.96' N 39°33.277'E., *leg.* B.A. Levin, 3 April 2016. Sample E14.

Description. LM (Figs 27–59). Cells in girdle view rectangular, slightly bent. Valves linear-elliptic to linear-lanceolate, with rounded ends. Length $8.6-11.7~\mu m$ ($9.8\pm0.6;~n=32$), width $3.7-4.5~\mu m$ ($4.1\pm0.2;~n=32$). Raphe straight, filiform, with expanded central ends. Axial area on the raphe valve narrow linear, central area may be indistinct, usually asymmetric, formed by 1-2 central striae shortened and spaced wider than the rest. Striae radiate, 15-16 in $10~\mu m$. Axial area on the rapheless valve also linear, widening towards the centre. A rounded cavum is present on the one

side of the rapheless valve. Striae weakly radiate to almost parallel in the centre of the valve, more radiate towards the ends, 14-18 in $10 \mu m$.



FIGURES 27–40. *Planothidium paisiusii sp. nov.* LM, DIC. Sample of wild population, size diminution series. Figs 27–33. Raphe valves. Figs 34–39. Rapheless valves. Fig. 40. Girdle view. Scale bar = 10 µm.



FIGURES 41–63. *Planothidium paisiusii sp. nov.* Strain CBMC155ef, type material. 41–59. LM, DIC. Slide no. 05583 from oxidized culture. 41–50. Raphe valves. 51–59. Rapheless valves. Holotype represented by fig. 49. 60–63. SEM. 60. Raphe valve, external view. 61. Rapheless valve, external view. 62. Raphe valve, internal view. 63. Rapheless valve, internal view. Scale bar 10 μm (figs 41–59), 2 μm (figs 60, 61), 1 μm (figs 62, 63).

SEM. Raphe valve (Figs 60, 62). External central raphe ends straight, expanded, distal ends curved to one side and extended onto the mantle. Internal central raphe ends slightly bent to the opposite sides and set quite widely apart, distal ends terminate in small helictoglossae. Striae multiseriate, each stria consists of 4 (3) rows of small circular areolae.

Rapheless valve (Figs 61, 63). Cavum large, rounded, usually with a relatively small opening. A vestigial raphe is present on the inside. Striae multiseriate, consisting of 3–4 rows of small circular areolae. Internally, the interstriae are distinctly raised on both valves.

Sequence data. Partial 18S rDNA gene sequence comprising V4 domain sequence (GenBank accession number PV383266) and partial *rbc*L sequence (GenBank accession number PV384975) for the strain CBMC155ef.

Etymology. This species is named after Archimandrite Paisius (Vasily Filippovich Balabanov, 1832–1912), the leader of the spiritual mission of Ashinov's expeditions to Abyssinia in 1888–1889, honoring his contribution to the strengthening of Russia-Abyssinia ties.

Distribution. As yet known only from the type locality.

Molecular study. On the phylogenetic tree (Fig. 64), this species forms a separate branch that is related to strains of the *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot (1999: 282) and *Planothidium victori* Novis, Braidwood & Kilroy in Novis *et al.* (2012: 22) complex.

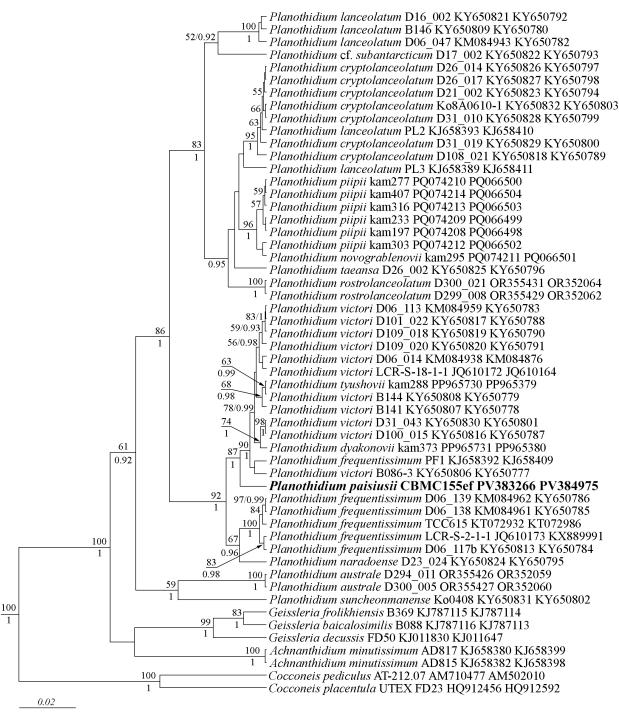


FIGURE 64. Phylogenetic position of *Planothidium paisiusii sp. nov.* (indicated in bold) based on Bayesian inference for a concatenated alignment of 54 partial *rbc*L and partial 18S rDNA sequences. The total length of the alignment is 1,040 characters. Values above the horizontal lines are bootstrap support from ML analyses (< 50 are not shown); values below the horizontal lines (or to the right of the slash) are Bayesian posterior probabilities (< 0.9 are not shown). Strain numbers (if available) and GenBank numbers are indicated for all sequences.

Discussion

Sellaphora ashinovii sp. nov.

Sellaphora ashinovii sp. nov. is morphologically close to S. saugerresii (Desmazières) C.E. Wetzel & D.G. Mann in Wetzel et al. (2015: 209) (see Table 3). These species are similar in valve width (3.5–4.5 μm in S. ashinovii sp. nov. and 3–4 μm in S. saugerresii) and in the shape of the central area (bowtie-shaped, formed by shortened striae). The main differentiating feature between these species is the striae structure: S. ashinovii sp. nov. is characterized by uniseriate striae, while in S. saugerresii the striae are always biseriate. The striae density also differs (24–26 in 10 μm in Sellaphora ashinovii sp. nov. vs. 18–22 in 10 μm in S. saugerresii). Another distinguishing trait is the shape of the axial area which is weakly widening towards the central area in Sellaphora ashinovii sp. nov. and linear in S. saugerresii.

TABLE 3. Morphological comparison of *Sellaphora ashinovii sp. nov.* and similar species.

	Sellaphora ashinovii sp. nov.	S. saugerresii	S. atomoides	S. subseminulum
Valve shape	linear-lanceolate with rounded ends	linear-elliptic or elliptic with rounded ends	elliptical to oval with rounded ends	lanceolate-elliptical with rounded ends
Valve length, µm	7.4–13.5	7–12	3.4–16.3	8.3-11.0
Valve width, µm	3.5–4.5	3–4	2.6-3.7	3.3–3.8
Axial area	narrow, weakly expanded towards the central area	narrow, linear	linear through most of the valve, slightly expanding near the central area	narrow*
Central area	bowtie-shaped, formed by 3–4 shortened striae on each side	rather large, transversely rectangular to weakly butterfly-shaped, not reaching the margins	wide, symmetrical and bowtie-shaped	rather large, transversely rectangular to weakly butterfly-shaped, formed by 5–6 very shortened striae (from one or two areolae) on each side
Striae pattern	uniseriate	biseriate	uniseriate	n.d.
Striae in 10 µm	24–26	18–22	30–36	20–24
Distribution	Holarctic	Holarctic	Holarctic	Göttingen, Germany
Reference	this study	Wetzel <i>et al.</i> 2015; Lange-Bertalot <i>et al.</i> 2017	Wetzel et al. 2015	Brendemühl 1949; Reichardt 2006; Wetzel <i>et al.</i> 2015; Foets & Wetzel 2018

^{*}derived from published illustrations.

S. atomoides (Grunow) Wetzel & Van de Vijver in Wetzel et al. (2015: 219) also resembles Sellaphora ashinovii sp. nov. (see Table 3) in valve shape, striae pattern, and structure of central and axial area, the central area being formed by shortened striae in both species. These species can be differentiated by valve width (3.5–4.5 μm in Sellaphora ashinovii sp. nov. vs. 2.6–3.7 μm in S. atomoides) and striae density (24–26 in 10 μm in Sellaphora ashinovii sp. nov. vs. 30–36 in 10 μm in S. atomoides).

Lastly, *Sellaphora ashinovii sp. nov.* is similar to *S. subseminulum* (Hustedt) C.E. Wetzel in Wetzel *et al.* (2015: 218) (see Table 3). These species resemble each other in valve shape, striae pattern, and shape of axial and central areas. They can be differentiated by valve width (3.5–4.5 μm in *Sellaphora ashinovii sp. nov.* vs. 3.3–3.8 μm in *S. subseminulum*). The axial area in the new species is narrow, widening weakly towards the central area, while in *S. subseminulum* the axial area does not widen (Wetzel *et al.* 2015, figs 180–182). The central area is bordered by 3–4 shortened striae in *Sellaphora ashinovii sp. nov.* and by 5–6 very short striae in *S. subseminulum* (see Table 3). The striae density is 24–26 in 10 μm in *Sellaphora ashinovii sp. nov.* and 20–24 in 10 μm in *S. subseminulum*.

On the phylogenetic tree, the strain of *S. ashinovii sp. nov.* forms a separate branch that is sister to strains of *Sellaphora minima* (Fig. 26). Unfortunately, there are no vouchers available for the strain TCC524 of *S. minima*, thus a morphological comparison with our new species cannot be carried out. Strain BM42, identified as *S. cf. minima*, was illustrated with a single image in Evans *et al.* (2008, Fig. 8c), which is also insufficient for a proper morphological

comparison. Even so, judging by the one available image, this strain differs from *S. ashinovii sp. nov.* by valve outline, size of central area, and striae density. Currently, according to Wetzel *et al.* (2015), *S. minima* is regarded as a synonym for *S. saugerresii* which was discussed above.

Representatives of *Sellaphora* Mereschkowsky have been fairly well studied with the use of morphological and molecular analysis (Evans *et al.* 2008; Mann *et al.* 2009; Vanormelingen *et al.* 2013, Wetzel *et al.* 2015), with new species being described in recent years from different parts of the world (Andreeva *et al.* 2018; Kochoska *et al.* 2021; Glushchenko *et al.* 2022; Ni *et al.* 2022 and others). Nevertheless, acquisition of molecular data from new strains from various geographical locations is essential for correct identification of taxa in floristic and ecological studies, as well as for taxonomical investigations, especially since the genus *Sellaphora* contains difficult species complexes, e.g. *Sellaphora pupula* (Mann *et al.* 2004; Evans *et al.* 2008, etc.).

Planothidium paisiusii sp. nov.

According to morphological and molecular data, the new species belongs to the genus *Planothidium*, characterized by a cavum on the rapheless valve, multiseriate striae and straight raphe branches with distal ends turned to one side and extended onto the mantle. The new species can be compared to such known species as *P. straubianum* Wetzel, Van de Vijver & Ector in Wetzel *et al.* (2019: 60), *P. aueri* (Krasske) Lange-Bertalot (1999: 275), and *P. curtistriatum* Wetzel, Van de Vijver & Ector in Wetzel *et al.* (2019: 61). *P. straubianum* (Wetzel *et al.* 2019) is the most similar to the new species; it can be differentiated by a more linear-elliptic valve outline and a small narrow cavum. The valve dimensions of the two species overlap, but the valves of *P. straubianum* are generally wider and have a higher maximum length as well (see Table 4). The axial area on the rapheless valve is linear in *P. straubianum*, more distinctly widened towards the centre in *P. paisiusii sp. nov.*; the striae on the rapheless valve in *P. straubianum* consist of 4–5 rows of areolae vs. 3–4 rows in *P. paisiusii sp. nov.* The outside of the rapheless valve in *P. straubianum* is ornamented with rounded depressions, while in *P. paisiusii sp. nov.* there is no ornamentation present.

TABLE 4. Morphological comparison of *P. paisiusii sp. nov.* and similar species.

	Planothidium paisiusii sp. nov.	P. aueri	P. curtistriatum	P. straubianum
Valve shape	linear-elliptic to linear-lanceolate	lanceolate	elliptic-lanceolate	elliptic-lanceolate with parallel sides
Valve apices	rounded	broadly rounded	rounded	rounded
Valve length, μm	8.6–11.7	11.5–25	5.5–14.5	6.0-14.0
Valve width, µm	3.7–4.5	3.0-4.5	3.5–5.5	4.0-5.5
Raphe valve				
Axial area	narrow linear	lanceolate	narrow	narrow, widening near the centre
Central area	indistinct, asymmetric	narrow rectangular, asymmetric	indistinct, squared	elliptic, asymmetrical
Striae pattern	radiate	radiate	weakly radiate	radiate
Rows of areolae in a stria	4(3)	2	3–4	3–4
Striae density in 10 μm	15–16	18–20	11–14	14–18
Rapheless valve				
Axial area	linear, widens towards centre	lanceolate	wide lanceolate	narrow linear
Central area	rounded cavum on one side	rounded cavum on one side	small cavum on one side	small narrow cavum on one side
Ornamentation	absent	n.d.	shallow round irregular depressions	small round depressions
Striae pattern	almost parallel in the centre, radiate towards the ends	weakly radiate	weakly radiate	weakly radiate
Rows of areolae in a stria	3–4	n.d.	3	4–5
Striae density in 10 μm	14–18	18–20	11–14	14–18
Reference	this study	Van de Vijver <i>et al</i> . 2002	Wetzel et al. 2019	Wetzel et al. 2019

P. curtistriatum (Wetzel et al. 2019) is also a small-celled species, however, it has a distinct large axial area on the rapheless valve that distinguishes it from other Planothidium species including P. paisiusii sp. nov. Other differentiating features between P. curtistriatum and the new species are the valve outline which is more lanceolate in P. curtistriatum, coarser striae (11–14 in P. curtistriatum vs. 15–16 on the raphe valve and 14–18 on the rapheless valve in P. paisiusii sp. nov.), and the ornamentation on the rapheless valve (rounded depressions in P. curtistriatum, absent in P. paisiusii sp. nov.).

P. paisiusii sp. nov. can be compared to smaller valves of P. aueri (Lange-Bertalot et al. 1996; Van de Vijver et al. 2002); there is resemblance in the cavum shape and the structure of the axial area on the rapheless valve. However, the valve outline of P. aueri is different from the new species and the quantitative features such as valve length and width and striae density also allow to differentiate these species (see Table 4): the valves of P. aueri are larger than P. paisiusii sp. nov. and the striae density is higher.

According to molecular data, *P. paisiusii sp. nov.* forms a separate branch in a subclade that includes several other species of *Planothidium* and is part of a larger clade containing *Planothidium* species. Mostly this subclade is formed by strains of *P. victori*; the identity of other strains in this subclade was previously discussed in Tseplik *et al.* (2024b). Our data supports the idea of *P. victori* actually representing a complex of several cryptic species. Morphologically, *P. victori* can be differentiated from the new species by its protracted ends, large wide cavum, linear axial area on the rapheless valve, and the ornamentation on the rapheless valve in the form of small round depressions; the quantitative features overlap between the two species, however, the valves of *P. paisiusii sp. nov.* are generally smaller (maximum length 11.7 µm vs. 19.5 µm in *P. victori* (Wetzel *et al.* 2019)). *Planothidium* also contains cryptic diversity and several complicated species groups (Jahn *et al.* 2017; Tseplik *et al.* 2024 a, b), and accumulation of DNA sequences of new strains is an important asset in resolving the taxonomy of this genus.

Our study is one of the first molecular studies of diatoms in Africa. Establishing a library of strains from different locations is a very important step in diatom research, and data like this is valuable for future studies in taxonomy, biodiversity, and biomonitoring.

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