

MORPHOLOGICAL AND MOLECULAR GENETIC ANALYSIS OF GENUS *VISCHERIA* (EUSTIGMATACEAE, OCHROPHYTA) IN THE ACSSI ALGOLOGICAL COLLECTION

A. D. Temraleeva^{a,*} and E. A. Portnaya^a

^a Institute of Physicochemical and Biological Problems in Soil Science of the Russian Academy of Science,
Federal Research Center “Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences”
Institutskaya Str., 2, Pushchino, 142290, Russian Federation

*e-mail: temraleeva.anna@gmail.com

DOI: 10.31857/S0006813622020119

Morphological and molecular genetic analysis of four strains of soil eustigmatophyte algae isolated from grey forest soils of Moscow and Tula regions of Russia and kept in the Algal Collection of Soil Science Institute (ACSSI) was carried out. According to the 18S rRNA and ITS2 phylogeny data, the studied strains are the members of the *Vischeria* genus. They are morphologically close to *V. magna*; however, only one ACSSI 026 strain with high statistical support clustered with the authentic SAG 2554 strain. The other strains formed a separate independent group. The paper shows that the taxonomy of the genus seems to be problematic due to the unresolved phylogenetic tree of 18S rRNA and ITS2, the lack of information content of the variable regions V4-V5 and V8-V9 of the 18S rRNA gene and the failure of the CBC approach based on the separation of closely related species in the presence of at least one CBC in the conservative regions of the secondary structure of ITS2. It is assumed that an increase in the number of strains of eustigmatophyte algae isolated from various biotopes, the use of plastid genes or deep sequencing of the whole plastid genome, the study of ultrastructural, physiological, and biochemical characteristics will make it possible to develop the concept of a species in eustigmatophyte algae in general and in the genus *Vischeria* in particular.

Key words: eustigmatophyte algae, light microscopy, phylogeny, 18S rRNA, ITS2, CBC approach

ACKNOWLEDGEMENTS

The work was supported by the Russian Science Foundation, project number 19-74-00030.

REFERENCES

- Amaral R., Fawley K.P., Nemcová Y., Sevcíková T., Lukesová A., Fawley M.W., Santos L.M.A., Eliáš M. 2020. Toward modern classification of eumastigophytes, including the description of Neomonodaceae fam. nov. and three new genera. — *J. Phycol.* 56 (3): 630–648. <https://doi.org/10.1111/jpy.12980>
- Byun Y., Han K. 2009. PseudoViewer3: generating planar drawings of large-scale RNA structures with pseudoknots. — *Bioinformatics.* 25 (11): 1435–1437. <https://doi.org/10.1093/bioinformatics/btp252>
- Bradley I.M., Pinto A.J., Guest J.S. 2016. Design and evaluation of Illumina MiSeq-compatible, 18S rRNA gene-specific primers for improved characterization of mixed phototrophic communities. — *Appl. Environ. Microbiol.* 82 (19): 5878–5891. <https://doi.org/10.1128/AEM.01630-16>
- Cepák V., Přibyl P., Kohoutková J., Kaštánek P. 2014. Optimization of cultivation conditions for fatty acid composition and EPA production in the eustigmatophycean microalga *Trachydiscus minutus*. — *J. Appl. Phycol.* 26 (1): 181–190. <https://doi.org/10.1007/s10811-013-0119-z>
- Coleman A.W. 2009. Is there a molecular key to the level of “biological species” in eukaryotes? A DNA guide. — *Mol. Phylogenet. Evol.* 50: 197–203. <https://doi.org/10.1016/j.ympev.2008.10.008>
- Coleman A.W. 2000. The significance of a coincidence between evolutionary landmarks found in mating affinity and a DNA sequence. — *Protist.* 151 (1): 1–9. <https://doi.org/10.1078/1434-4610-00002>
- Durmaz Y. 2007. Vitamin E (α -tocopherol) production by the marine microalgae *Nannochloropsis oculata* (Eustigmatophyceae) in nitrogen limitation. — *Aquaculture.* 272: 717–722. <https://doi.org/10.1016/j.aquaculture.2007.07.213>
- Eliáš M., Amaral R., Fawley K.P., Fawley M.W., Nemcova Y., Neustupa J., Přibyl P., Santos L.M.A., Sevcikova T. 2017. Eustigmatophyceae. — In: Handbook of the Protists. Archibald J.M., Simpson A.G.B., Slamovits C.H. [Eds.] Springer International Publishing. Switzerland. P. 367–406.
- Ettl H., Gärtner G. 1995. Syllabus der Boden-, Luft-, und Flechtenalgen. Stuttgart, Jena, New York, Gustav Fischer. 710 p.
- Fawley K.P., Eliáš M., Fawley M.W. 2014. The diversity and phylogeny of the commercially important algal class Eustigmatophyceae, including the new clade Goniochloridales. — *J. Appl. Phycol.* 26: 1773–1782. <https://doi.org/10.1007/s10811-013-0216-z>
- Fukuda S.Y., Iwamoto K., Atsumi M., Yokoyama A., Nakayama T., Ishida K., Inouye I., Shiraiwa Y. 2014. Global searches for microalgae and aquatic plants that can eliminate radioactive cesium, iodine and strontium from the radio-polluted aquatic environment: A bioremediation strategy. — *J. Plant Res.* 127 (1): 79–89. <https://doi.org/10.1007/s10265-013-0596-9>
- Gao B., Xia S., Lei X., Zhang Z. 2018. Combined effects of different nitrogen sources and levels and light intensities on growth and fatty acid and lipid production of oleaginous eustigmatophycean microalga *Eustigmatos*

- cf. *polyphem*. – J. Appl. Phycol. 30: 215–229.
<https://doi.org/10.1007/s10811-017-1180-9>
- Gao B., Yang J., Lei X., Xia S., Li A., Zhang C. 2016. Characterization of cell structural change, growth, lipid accumulation, and pigment profile of a novel oleaginous microalga, *Vischeria stellata* (Eustigmatophyceae), cultured with different initial nitrate supplies. – J. Appl. Phycol. 28: 821–830.
<https://doi.org/10.1007/s10811-015-0626-1>
- Guiry M.D., Guiry G.M. 2021. AlgaeBase. World-wide electronic publication. National University of Ireland. Galway. <http://www.algaebase.org>
- Hibberd D.J. 1981. Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribo-phyceae (synonym Xanthophyceae). – Bot. J. Linn. Soc. 82 (2): 93–119.
<https://doi.org/10.1111/j.1095-8339.1981.tb00954.x>
- Hibberd D.J., Leedale G.F. 1971. A new algal class – The Eustigmatophyceae. – Taxon. 20 (4): 523–525.
<https://doi.org/10.1038/225758b0>
- Katana A., Kwiatowski J., Spalik K., Zakryś B., Szalacha E., Szymańska H. 2001. Phylogenetic position of *Koliella* (Chlorophyta) as inferred from nuclear and chloroplast small subunit rDNA. – J. Phycol. 37 (3): 443–451.
<https://doi.org/10.1046/j.1529-8817.2001.037003443.x>
- Kryvenda A., Rybalka N., Wolf M., Friedl T. 2018. Species distinctions among closely related strains of Eustigmatophyceae (Stramenopiles) emphasizing ITS2 sequence-structure data: *Eustigmatos* and *Vischeria*. – Eur. J. Phycol. 53 (4): 471–491.
<https://doi.org/10.1080/09670262.2018.1475015>
- Li Z., Sun M., Li Q., Li A., Zhang C. 2012. Profiling of carotenoids in six microalgae (Eustigmatophyceae) and assessment of their β -carotene productions in bubble column photobioreactor. – Biotechnol. Lett. 34 (11): 2049–2053.
<https://doi.org/10.1007/s10529-012-0996-2>
- Martins C.B., Ferreira O., Rosado T. et al. 2021. *Eustigmatophyte* strains with potential interest in cancer prevention and treatment: partial chemical characterization and evaluation of cytotoxic and antioxidant activity. – Biotechnol. Lett.
<https://doi.org/10.1007/s10529-021-03122-0>
- Müller T., Philippi N., Dandekar T., Schultz J., Wolf M. 2007. Distinguishing species. – RNA. 3 (9): 1469–1472.
<https://doi.org/10.1261/rna.617107>
- Norton T.A., Melkonian M., Andersen R.A. 1996. Algal biodiversity. – Phycologia. 35: 308–326.
<https://doi.org/10.2216/i0031-8884-35-4-308.1>
- Pal D., Khozin-Goldberg I., Didi-Cohen S., Solovchenko A., Batushansky A., Kaye Y., Sikron N., Samani T., Fait A., Boussiba S. 2013. Growth, lipid production and metabolic adjustments in the euryhaline eustigmatophyte *Nannochloropsis oceanica* CCALA 804 in response to osmotic downshift. – Appl. Microbiol. Biotechnol. 97 (18): 8291–8306.
<https://doi.org/10.1007/s00253-013-5092-6>
- Patterson G.W., Tsitsa-Tzardis E., Wikfors G.H., Smith B.C., Gladu P.K. 1994. Sterols of Eustigmatophytes. – Lipids. 29 (9): 661–664. <https://doi.org/10.1007/BF02536102>
- Procházková K. 2012. Diversity and species concept of the *Vischeria/Eustigmatos* complex (Eustigmatophyceae): Graduation thesis. Praha. Karlova univerzita. 79 p. (In Czech.)
- Remias D., Nicoletti C., Krennhuber K. et al. 2020. Growth, fatty, and amino acid profiles of the soil alga *Vischeria* sp. E71.10 (Eustigmatophyceae) under different cultivation conditions. – Folia Microbiol. (Praha). 65 (6): 1017–1023.
<https://doi.org/10.1007/s12223-020-00810-8>
- Safiullina L.M., Muratova K.R., Zakirova M.B. 2014. Comparative analysis of microscopic soil algae *Eustigmatos magnus* and *Vischeria helvetica* (Eustigmatophyta). – Algologia. 24 (3): 270–273 (In Russ.).
- Stoykova P., Stoyneva-Gärtner M., Uzunov B., Gärtner G., Atanassov I., Draganova P., Borisova C. 2019. Morphological characterization and phylogenetic analysis of aeroterrestrial *Vischeria/Eustigmatos* strains with industrial potential. – Biotechnol. Biotechnol. Equip. 33 (1): 231–242.
<https://doi.org/10.1080/13102818.2018.1561212>
- Stoyneva-Gärtner M., Uzunov B., Gärtner G., Borisova C., Draganova P., Radkova M., Stoykova P., Atanassov I. 2019. Current bioeconomical interest in stramenopilic Eustigmatophyceae: a review. – Biotechnol. Biotechnol. Equip. 33 (1): 302–314.
<https://doi.org/10.1080/13102818.2019.1573154>
- Upadhyay A.K., Singh N.K., Singh R., Rai U.N. 2016. Amelioration of arsenic toxicity in rice: Comparative effect of inoculation of *Chlorella vulgaris* and *Nannochloropsis* sp. on growth, biochemical changes and arsenic uptake. – Ecotoxicol. Environ. Saf. 124: 68–73.
<https://doi.org/10.1016/j.ecoenv.2015.10.002>
- Wang F., Gao B., Huang L., Su M., Dai C., Zhang C. 2018. Evaluation of oleaginous eustigmatophycean microalgae as potential biorefinery feedstock for the production of palmitoleic acid and biodiesel. – Bioresour. Technol. 270: 30–37.
<https://doi.org/10.1016/j.biortech.2018.09.016>