ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“
БИОЛОГИЧЕСКИ ФАКУЛТЕТ
Книга 2 – Ботаника
Том 99

ANNUAL OF SOFIA UNIVERSITY “ST. KLIMENT OHRIDSKI”
FACULTY OF BIOLOGY
Book 2 – Botany
Volume 99

AEROPHYTIC GREEN ALGAE, EPIMYCOTIC ON *FOMES FOMENTARIUS* (L. EX FR.) KICKX.

MAYA P. STOYNEVA[1*], BLAGOY A. UZUNOV[1] & GEORG GÄRTNER[2]

1 – Sofia University “St. Kliment Ohridski”, Faculty of Biology, Department of Botany, 8 bld. Dr. Zankov, BG-1164, Sofia, Bulgaria; mstoyneva@abv.bg; blagoy_uzunov@abv.bg

2 – Institut für Botanik der Universität, Sternwartestraße 15, A-6020 Innsbruck, Österreich; georg.gaertner@uibk.ac.at

The paper is dedicated to Prof. D. Temnsikova on the occasion of her 80th jubilee

Abstract: Four species of green algae were found on the upper surface of *Fomes fomentarius* (L. ex Fr.) Kickx. basidiome. *Desmococcus vulgaris* (represented by both vegetative cells and aplanosporangia) dominated among them. *Trebouxia arboricola* (represented by free-living vegetative cells and autosporangia), and two species of *Stichococcus* (*S. bacillaris*, *S. minutus*) were the other identified algae. It is proposed to use the term “epimycotic” (from the greek “epi”- over and “mykes” – fungus) for algae and/or other organisms capable of growing and developing on the upper surface of fungal fruiting bodies.

Key words: basidiocarp, basidiome, Bulgaria, coccal algae, Danube island, filamentous algae, fungal host

* c*orresponding author*: M. P. Stoyneva – Sofia University “St. Kliment Ohridski”, Faculty of Biology, Department of Botany, 8 bld. Dr. Zankov, BG-1164, Sofia, Bulgaria; mstoyneva@abv.bg

INTRODUCTION

Aerophytic algae are well known colonists on different substrates as bark, wood, rocks, buildings, *etc.* and occur also as epiphytes on living organisms (*e.g.* on leaves of trees and shrubs, coniferous needles, lichens, *etc*. – Ettl & Gärtner 1995, 2014). Fruiting bodies of fungi have very rarely been reported to host algae on their surface and among them only a few species were identified (Burdsall et al. 1996; Zavada & Simoes 2001). Moreover, it was underlined that the occurrence of "epiphytes" on *Trametes versicolor* (L.) Lloyd is common, but not universal (Zavada et al. 2004). The recent investigation of an old, intermediate between bracket- and hoof-like, fruit body of *Fomes fomentarius* (L. ex Fr.) Kickx., collected in a floodplain Danube forest, revealed different green algae growing abundantly on its upper surface. Obviously, the air moisture of the site favors the development of the aerophytic algae found. For the algae and other organisms capable of growing and developing on such substrate, the term "epimycotic" is advocated.

MATERIAL AND METHODS

Fomes fomentarius from a *Populus* sp. trunk was collected in the middle of July 2014, in a floodplain forest at the Bulgarian Danube island Tsibur (=Ibisha). The island is situated between 716 and 719 river kilometers (Montana district, Bulgaria – Fig. 1.) and covers area of 0,9 km^2. In spite of this small territory, it is very interesting from nature conservational point of view and contains two protected territories: the protected area "Ostrov Tsibur" (situated in north-western part of the island with area of 101,48 ha, declared by State Order RD-292/10.04.2007) and managed reserve "Ibisha" (situated in the south-eastern part of the island with area of 34,3 ha, declared by Order RD-394/15.10.1999 of the Bulgarian Ministry of Environment and Waters). The "Ibisha" reserve is periodically inundated and contains floodplain forest. The *Fomes* fruiting body (also basidiocarp, basidiome, basidioma, fruit-body – after Kirk et al. 2008) was collected by Assoc. Prof. Dr. P. Mitov during his work on the Management Plan of the managed reserve "Ibisha" and on the next day transported to the Botany Department of Sofia University "St. Kliment Ohridski".

The basidiome was about 55 cm in diameter and up to 22 cm thick (measured on the highest part from top to basis) – Figs. 2a-b. The upper surface was tough, bumpy, hard and woody, mainly greyish. It was covered with many spots – light to dark-green, rounded or irregular in shape, 3 mm to 3 cm, and even in bigger size (Figs. 2a-b). Samples from six spots (marked with different coloured mini-flags and numbers – Fig. 2b) were taken with sterile needles and transferred directly to slides for investigation in light microscope. Microscopy was done with a Motic 400 microscope (40x and 100x immersion objectives). Photomicrographs were taken with a Motic Cam 2,0 and processed with software Motic Images Plus 2,0. Cell walls were stained with Methylene Blue and starch was coloured with Lugol's

solution (ETTL & GÄRTNER 1995, 2014). The taxonomic identification followed ETTL & GÄRTNER (2014).

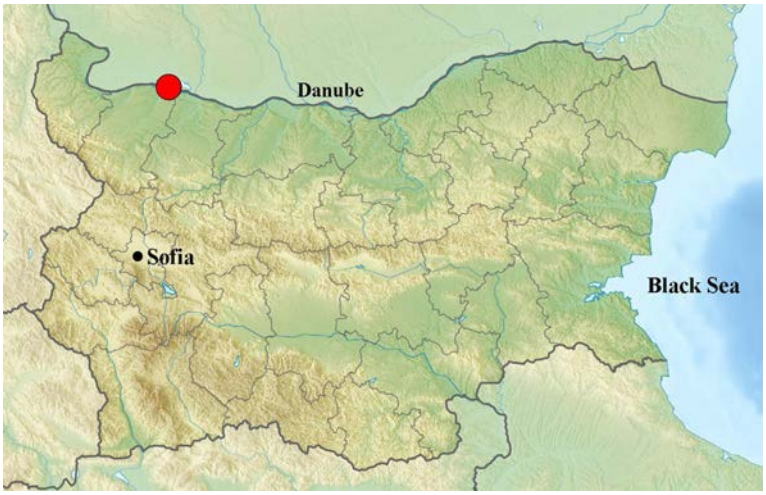

Fig. 1. Map of Bulgaria with the location of the managed reserve “Ibisha”, and *Fomes fomentarius*, respectively.

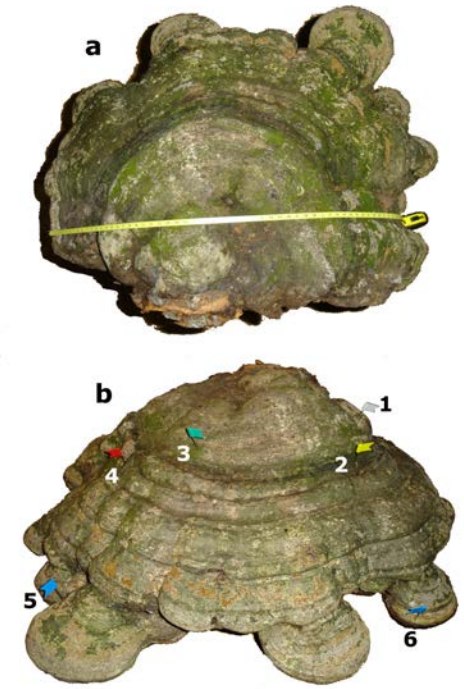

Fig. 2a-b: a) Basidiome of *Fomes fomentarius* with dark-green spots of epimycotic green algae; b) Locations of sampling points, marked with small coloured flags and numbers (1–6).

RESULTS AND DISCUSSION

Different live stages of the most common aerophytic green alga *Desmococcus olivaceus* (Pers. ex Ach.) Laundon (Trebouxiophyceae, Prasiolales) were found in all investigated samples from the upper surface of the basidiome. The alga formed 2–4-celled cuboidal packets with short unbranched filaments of 3–4 cells (Figs. 3a-d, 4a-c). The cells were rounded, relatively compressed in filamentous stages (Fig. 3a, 4c), with a well-developed, even massive, wall. The parietal chloroplast (one per cell) with irregularly lobed margin contained a small pyrenoid, covered with a fine starch sheath (visible when stained with Lugol's solution). Additionally, aplanosporangia with thick irregular cell walls, were recorded in one of the samples (Fig. 3d). Dimensions of vegetative cells in the few-celled filaments varied from 7 to 12 μm. *D. olivaceus* was firstly reported for Bulgaria as a distinct taxon (from cultures) by Stoyneva & Gärtner (2009), who recorded it from moist rock surfaces of the tunnel cave "Prohodna" and from an old glass piece, found in the entrance of the same cave. Most probably, it was found earlier in Bulgaria by Petkoff (1925) on tree barks in Pirin Mts. and included in the algal flora of Vodenicharov et al. (1971) under the unclear name *Protococcus viridis* Agardh, which led to taxonomic confusions (Uzunov et al. 2008).

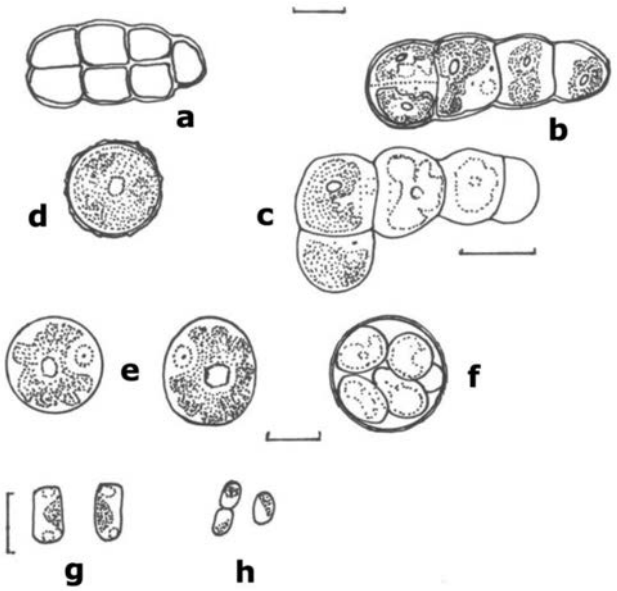

Fig. 3a-f. Epimycotic algae on *Fomes fomentarius*. 3a-d: *Desmococcus olivaceus*: a-c) Vegetative stages of cuboidal cell packets and short filaments; d) Aplanosporangium of *Desmococcus olivaceus* with thick cell wall. 3e-f: *Trebouxia arboricola*: e) vegetative cells with nucleus in the sinus of chloroplast; f) autosporangium with 8 autospores of different size. 3g-h: *Stichococcus*: g) vegetative cells of *St. bacillaris*; h) vegetative cells of *St. minutus.* Scale bars on the figs. – 10 μm.

Trebouxia arboricola Puymaly (Trebouxiophyceae, Trebouxiales) was detected in two of the surface samples (green and light-blue flags /= sites 3 and 6 on Fig. 2b). It was non-lichenized, free-living and appeared in both stages of vegetative cells and autosporangia (Figs. 3e-f, 4c–d). The vegetative cells possessed all typical diagnostic features of *Trebouxia aboricola*: they were globular, sometimes slightly ellipsoidal, with a parietal massive, lobed to incised chloroplast with a naked pyrenoid, which lacks a distinct starch sheath. The nucleus was eccentric in the cell lumen, situated in an expressed sinus of the chloroplast (Fig. 3e, 4e). The cells were 13–15 μm in diameter and coincided with the authentic strain (or "type culture") investigated by Gärtner (1975). The observed autosporangia contained mainly 8 autospores of slightly different size (Fig. 4f). *T. arboricola* was reported for Bulgaria by Vodenicharov et al. (1971) as found on tree bark in Rodopi Mts, without mentioning of cultures. Later on, it was collected in a free-living stage and cultivated from granite stone monuments (Gärtner & Stoyneva 2003), from the walls of the tunnel cave "Prohodna" (Stoyneva & Gärtner 2009) and from the surface of sandstones of the rock phenomenon "Belogradshishki Skali" (Mancheva 2013).

Two species of the very common aerophytic green filamentous genus *Stichococcus* Nägeli (Trebouxiophyceae, Prasiolales) were found in one of the studied samples (green flag/=site 3 on Fig. 2b): *S. bacillaris* Nägeli and *S. minutus* Grintzesco & Péterfi. Both algae differ in size and morphology of chloroplasts in their vegetative cells (Hindák 1996). The cells of *S. bacillaris* were ± cylindrical, 3μm broad and 7 (10) μm long, each with a median inserted chloroplast (Fig. 3g, 4d). By contrast, the vegetative cells of *S. minutus* were more rounded, 2–3μm broad and 4μm long, with a polar chloroplast within the cell lumen (Fig. 3h, 4d). *Stichococcus bacillaris* was recorded in many sites of Bulgaria (Vodenicharov et al. 1971; Uzunov et al. 2007, 2008), whereas *S. minutus* was found only by Uzunov (2009) in soils of Pirin Mts.

The recent findings are the first documented observations of *Desmococcus olivaceus*, *Trebouxia arboricola*, *Stichococcus bacillaris* and *S. minutus* as "epiphytic" algae on a basidiome surface in Bulgaria. According to our knowledege, this is also their first documentation on *Fomes fomentarius*, whereas "*Characium* sp. and *Coccomyxa* sp." were found on *Bridgeoporus nobilissimus* (W.B. Cooke) Volk, Burdsall & Ammirati (Burdsall et al. 1996), "*Hormidium* sp., *Stichococcus bacillaris*, *Chlorococcum* sp., and *Trebouxia* sp." were determined on *Trametes versicolor* and it was suggested that the basidiocarps of *T. versicolor* have the potential to be lichenized (Zavada et Simoes 2001). Later on, Zavada et al. (2004) proved the capacity for *T. versicolor* to exploit algae as a carbon source. We believe that many algologists, mycologists and ecologists know the possibility algae (and particularly green algae) to develop on the surface of different fungi. However, it is obvious that this so-well-known knowledge remained very less documented, at least in the literature available and checked for this study. In any case, we found illogical to continue to use the term "epiphytes" for the organisms from such surfaces, since long-ago it is clear that fungi do not belong to the Plant

Kingdom. Therefore, we plead to use for algae and/or other organisms growing on the upper surface of fungal fruiting bodies, the term "epimycotic" (from the greek "*epi*"-over and "*mykes*" – fungus), which occasionally and without discussions has been used (e.g. REYNOLDS 1978; REDBERG et al. 2003).

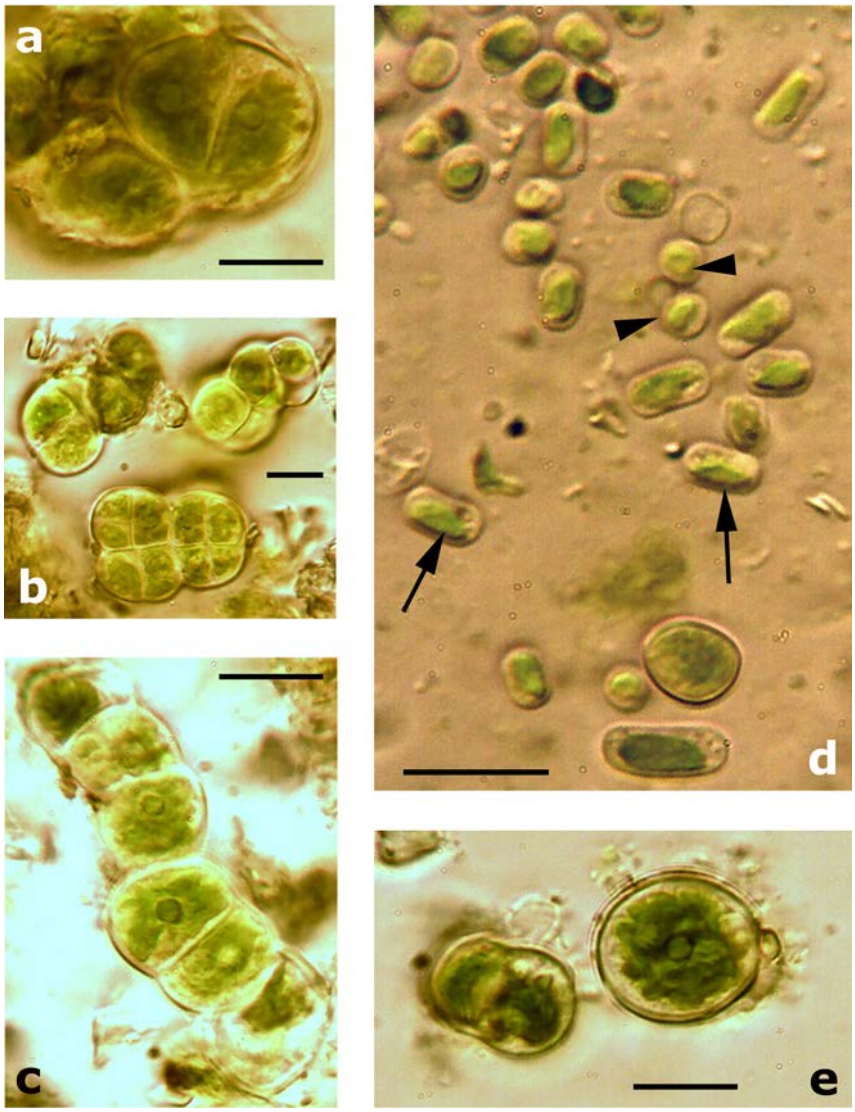

Fig. 4a-f. Photomicrographs of epimycotic algae on *Fomes fomentarius*. 4a-c: *Desmococcus olivaceus*: a) Single cells in binary division; b) Cuboidal packages of vegetative cells; c) Short filaments. 4d: *Stichococcus*: vegetative cells of *S. bacillaris* (arrow heads) and of *S. minutus* (arrows). 4e: *Trebouxia arboricola* – vegetative cells. Scale bars on the figs. – 10 µm.

REFERENCES

Burdsall H. H., Volk T. J. & J. F. Ammirati Jr. 1996. *Bridgeoporus*, a new genus to accommodate *Oxyporus nobilissimus* (Basidiomycota, Polyporaceae). – Mycotaxon 60: 387–395.

Ettl H. & Gärtner G. 1995. Syllabus der Boden-, Luft-und Flechtenalgen, Fischer, Stuttgart, Jena, New York, 721 pp.

Ettl H. & Gärtner G. 2014. Syllabus der Boden-, Luft-und Flechtenalgen. 2. Aufl., Springer, Berlin, Heidelberg, 773 pp.

Gärtner G. 1975. Die Gattung *Trebouxia* Puymaly. – Algol. Stud. 41: 495–548.

Gärtner G. & Stoyneva M. P. 2003. First Study of Aerophytic Cryptogams on Monuments in Bulgaria.- Ber. nat.- med. Verein Innsbruck 90: 73–82.

Hindák F. 1996. Klúč na určovanie nerozkonárených vláknitých zelených rias (Ulotrichineae, Ulotrichales, Chlorophyceae) [Key to unbranched filamentous green algae (Ulotrichineae, Ulotrichales, Chlorophyceae)]. – Bull. Slov. Bot. Spol., Bratislava, Suppl. 1: 1–77 (In Slovakian).

Kirk P. M., Cannon P. F., Minter D. W. & Stalpers J. A. (eds.) 2008. Ainsworth and Bisby's Dictionary of the Fungi. Tenth Edition prepared by CABI Europe – UK, 784 pp.

Mancheva A. 2013. Aerophytic algae from the rock phenomenon and nature monument Belogradchishki Skali. PhD Thesis, Sofia Univrsity, Fac. Biology, 191 pp. (In Bulgarian).

Petkoff S. 1925. La flore algologique du mont Pirin-planina. – Sbornik na Bulgarskata Akademiya na Naukite 20: 1–128 (In Bulgarian).

Redberg G. L., Hibbert D. S., Ammirati jr J. F. & Rodriguez R. J. 2003. Phylogeny and genetic diversity of *Bridgeoporus nobilissimus* inferred using mitochondrial and nuclear rDNA sequences. – Mycologia 95 (5): 836–845.

Reynolds D. R. 1978. Foliculous Ascomycetes 1: The Capnodiaceous genus *Scorias*, Reproduction. – Contributions in Science, Natural History Museum of Los Angeles 288: 1–15.

Stoyneva M. P. & Gärtner G. 2009. Remarkable and newly recorded aeroterrestric Cyanoprokaryotes and algae in Bulgaria. – In: Ivanova D. (ed.), Plant, fungal and Habitat diversity investigation and conservation. Proceedings of IV Balkan Botanical Congress, Sofia, 20–26 June 2006. Institute of Botany, Sofia, 122–127.

Uzunov B. A. 2009. Aeroterrestrial algae from Pirin Mountain (Bulgaria). PhD Thesis, Innsbruck University, 182 pp.

Uzunov B. A., Stoyneva M. P. & Gärtner G. 2007. Review of the studies on aero-terrestrial cyanoprokaryotes and algae in Bulgaria with a Checklist of recorded species. I. – Phytol. Balcan. 13 (1): 65–73.

Uzunov B. A., Stoyneva M. P. & Gärtner G. 2008. Review of the studies on aero-terrestrial cyanoprokaryotes and algae in Bulgaria with a Checklist of the recorded species. II. – Phytol. Balcan. 14: 11–18.

Vodenicharov D., Draganov S. J. & Temniskova D. 1971. Flora of Bulgaria. Algae. Izd. Narodna Prosveta, Sofia, 642 pp. (In Bulgarian).

Zavada M. S. & Simoes P. 2001. The possible demi-lichenization of the basidiocarps of *Trametes versicolor* (L.: Fries) Pilat (Polyporaceae). – Northeastern Naturalist 8 (1): 101–112.

Zavada M. S., DiMichele L. & Toth C. R. 2004. The demi-lichenization of *Trametes versicolor* (L.: Fries) Pilat (Polyporaceae): The transfer of fixed $^{14}CO_2$ to *T. versicolor*. – Northeastern Naturalist 11 (1): 33–40.

Accepted 26 November 2014