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First Study of Aerophytic Cryptogams on Monuments in Bulgaria

by

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Synopsis: The first study of aerophytic cryptogams from granite monuments investigated in the Bulgarian town Koprivshtitsa (Sredna Gora Mountains – 1058 m a.s.l.) compared with two identical bronze and limestone copies in the capital town Sofia (650 m a.s.l.) is presented. Greenish layers on the surface of one of the Koprivshtitsa monuments consisted of the aerophytic green alga *Apatococcus lobatus* together with free-living and lichenized green alga *Trebouxia arboricola* and the aerophytic green alga *Coccomyxa* sp. Some lichens as *Lepraria* cf. *neglecta* and *Candelariella vitellina* were also found. The same lichens together with further species like *Protoparmeliopsis muralis* var. *muralis* and *Caloplaca* sp. were detected on the surrounding stonework of the second Koprivshtitsa monument. On the surface of the calcareous statue in Sofia only some initial stages of *Apatococcus lobatus* were registered. The algal taxa have been studied in cultures and some comments on their taxonomy and distribution are given.

Key words: aerophytic algae, lichens, granite, limestone, *Apatococcus lobatus*, *Trebouxia arboricola*, Bulgaria.

1. Introduction:

Nevertheless that the algal, fungal, lichen and moss flora of Bulgaria has been well studied since many decades, the aerophytic cryptogams on monuments have not been investigated (VODENICHAROV et al. 1993; DENCHEV & BAKALOVA 2002) except one unpublished study of the World Cultural Heritage site Madarski Konnik (DRAGANOV et al. 1993). Biodeterioration of calcareous stones and marble caused by cyanoprokaryotes, algae, fungi and lichens, as well as the phycoflora on granite monuments are documented from different parts of Europe (e.g. NIMIS et al. 1992, PANTAZIDOU & ROUSSOMOUSTAKAKI1996, NOGUEROL-SEOANE & RIFON-LASTRA 1997, KOVÁČIK 2000, SICKS et al. 1991, DARIENKO 2002, UHER & KOVÁČIK 2002). In all these studies attention to the taxonomy of the detected aerophytes was also paid.

This paper presents the results from the first investigation of aerophytes on granite and limestone monuments in Bulgaria with use of algal cultures. The occurrence of *Apato-*

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coccus lobatus (CHODAT) J.B. PETERSEN and free-living *Trebouxia arboricola* PUYMALY in Bulgaria is proved and new localities for these species are documented. The four detected lichens, known as widespread in Bulgaria, are reported for the first time from monument surfaces in the country.

2. Material and methods:

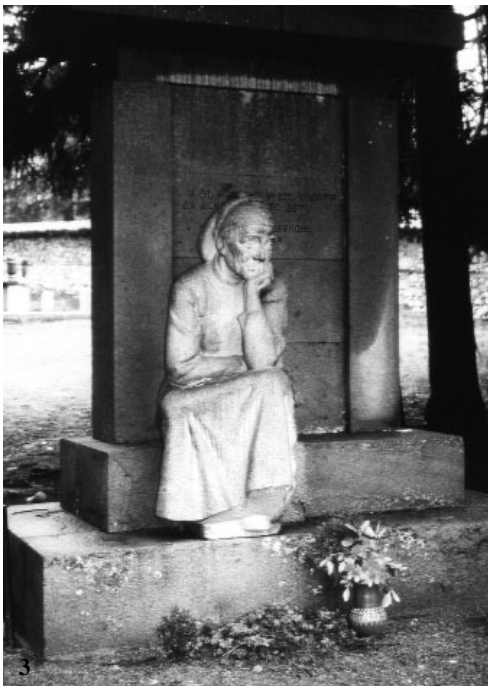
The samples were collected on 13.04.2003 from the granite monument "The Debelyanovs' Mother" situated in the garden of the museum of the poet Dimcho Debelyanov in the town of Koprivshtitsa (1058 m a.s.l.). Macroclimatic conditions are transitional – continental with the lowest average temperature in January (-4.3 °C), the highest average temperature in July (16.9 °C) and the mean annual precipitation of 748 mm (MICHEV et al. 1980; NIKOLOV & YORDANOVA 1997). The monument was erected in 1934 and actually is situated in a deep shadow in a planted grass parcel below *Picea abies* (L.) KARST. with an age of about 100 years and is surrounded by several small shrubs of *Buxus sempervirens* L. According to the personal information from the curator of the museum the first visible green layers of microorganisms on the granite surface appeared about 20 years ago. Later on the museum staff 3-4 times cleaned the monument. Actually the granite figure is covered again by powdery green layers of algae and some green or whitish-grey spots of lichens of different age and stage of development (Figs. 1, 2). Samples of this macroscopically observed material were carefully scraped with a knife and collected in aseptical plastic bags. Well-developed lichens could not be sampled without destroying the monument surface and were therefore documented only photographically. The bark of *Picea abies* beside the monument was also overgrown by green aerophytes and material from them was scraped for comparison.

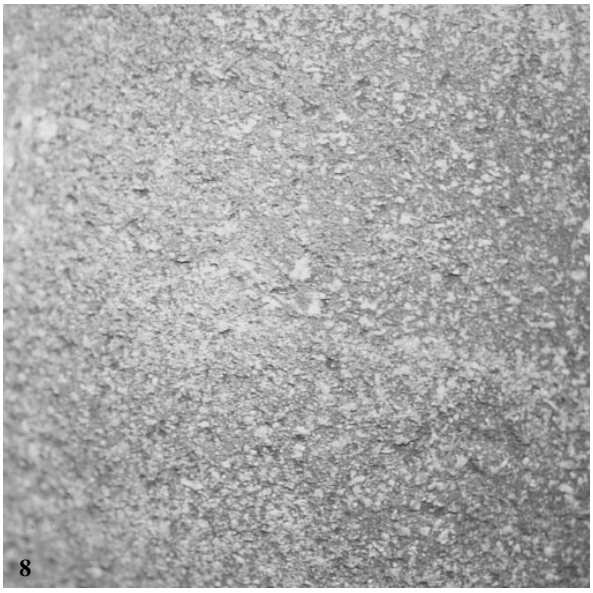
An identic copy of the museum monument but with a granite wall on its backside exists in a sun-exposed place on the grave of the poet in the nearby-located churchyard of Koprivshtitsa (Fig. 3). On the figure's surface no macroscopically visible cryptogams could be found. Aerophytic algae and lichens were there restricted to the basal part and the surrounding stonework (Figs. 3, 4), from where samples were scraped and collected.

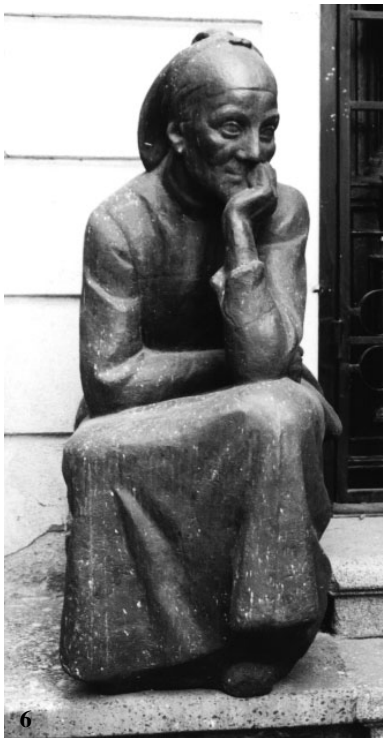
Another dark-bronze copy of this monument was erected in 1985 in the central part of the town of Sofia (650 m a.s.l.), nearby one of the main streets (boulevard Vasil Levski) in front of the house-museum of its author - the sculptor Ivan Lazarov. This copy was never overgrown by aerophytes. The white spots and strips documented during the visit on 26.04.2003 were from the colour splashes still not cleaned after the repairing of the facade of the nearby house (Figs. 5, 6). One more copy of the same statue made from limestone "Vrachanski kamuk" was erected in 2001 in the garden of National Art Gallery in Sofia on Moskovska street in a shady planted grass parcel below *Sambucus nigra* L. and *Robinia pseudacacia* L. (Fig. 7). Some small greyish-green algal layers were observed macroscopically on one side of the statue (Fig. 8). This material was collected on 9.06.2003 and processed in the same way as the material from Koprivshtitsa monuments. Macroclimatic conditions are temperate – continental with the lowest average temperature in January (-3°C), the highest average temperature in July-August (21-24°C) and the mean annual precipitation of 685 mm (DIMITROV & VELEV 1982; TISHKOV & VEKILSKA 1982).

A part of the algal material was observed immediately after sampling with a light microscope Amplival-Jena with highest magnification 1200x. Another part was cultivated on BBM-agar following ETTL & GÄRTNER (1995). Herbarium material is stored in the collection of the Department of Botany, Sofia University "St. Kliment Ohridski" (Bulgaria). Algal cultures are deposited in the culture collection of algae at the Botanical Institute of the University of Innsbruck (Austria). Micrographs and drawings were made using a light microscope Olympus BH-2 and an Olympus PM-10AK photomicrograph system.

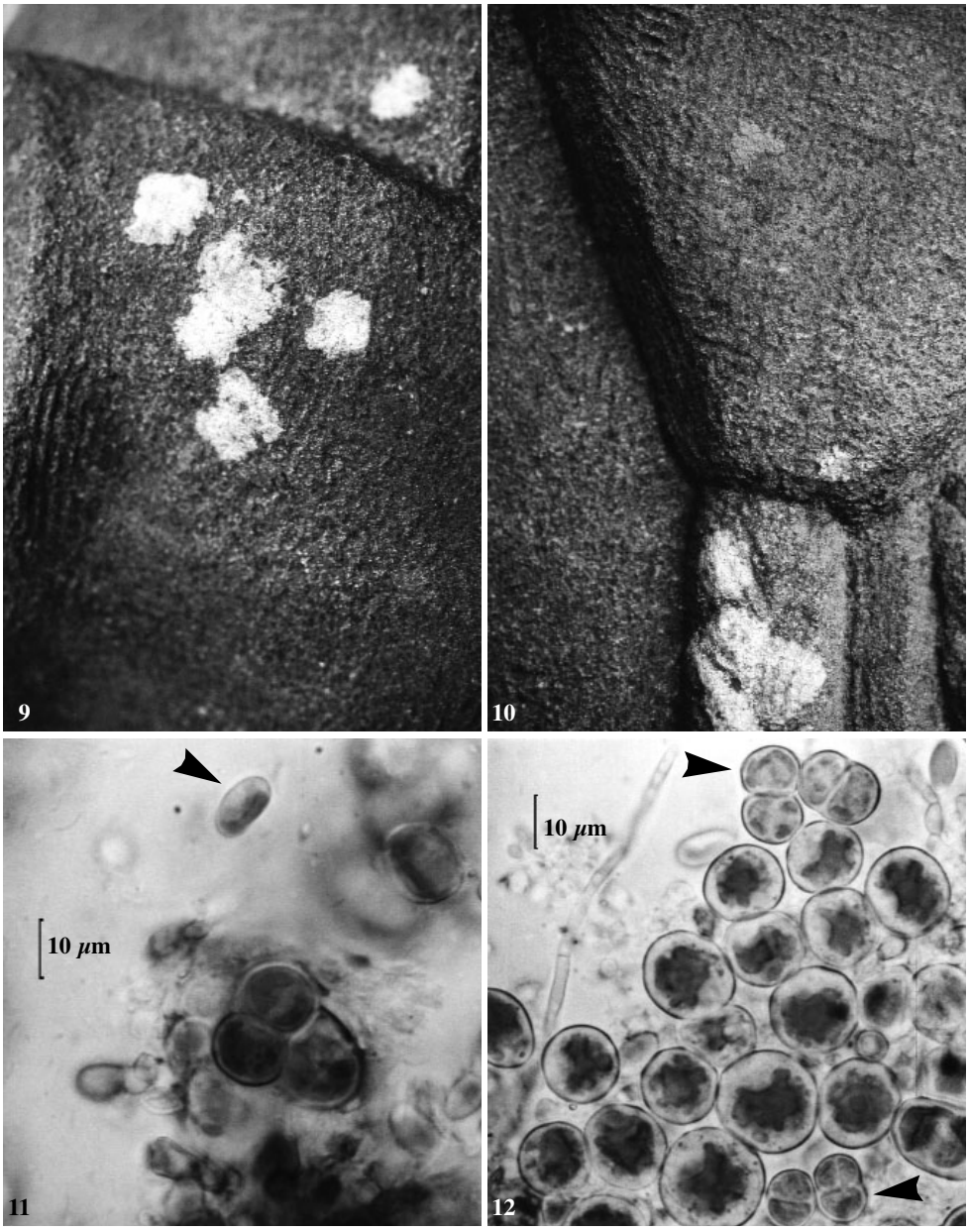
Figs. 1-4:
1 General view of the granite monument " The Debelyanovs' mother" at the museum of D. Debelyanov in Koprivshtitsa; **2** algal layers and lichens covering the monument's surface; **3** granite monument on D. Debelyanovs' grave in the churchyard of Koprivshtitsa, aerophytes covering only the base of the surrounding stonework; **4** fragment of this stonework with lichens (yellow: *Candelariella vitellina*, gray-green: *Protoparmeliopsis muralis* var. *muralis*).



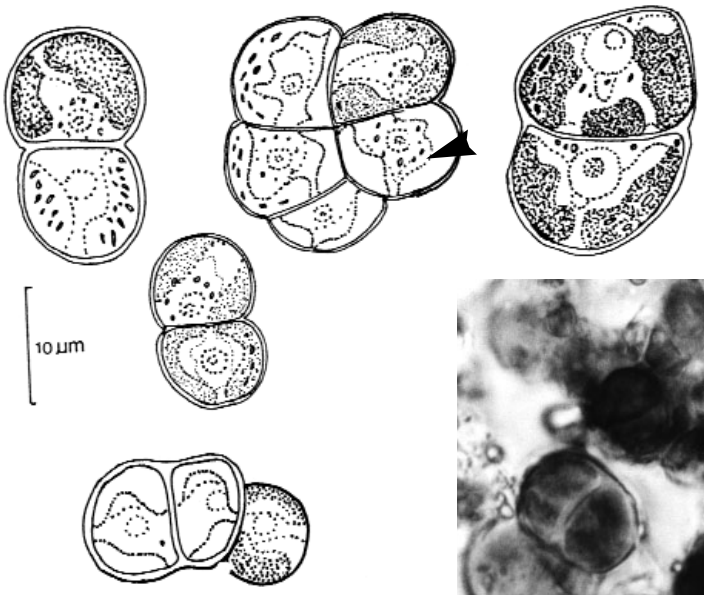




Figs 5 - 8:
5 General view of the bronze monument copy in front of Ivan Lazarov Museum in Sofia; **6** surface of the bronze monument without aerophytes, gray pricks of artifical coloration; **7** limestone copy of the Debelyanovs' mother monument near the National Art Gallery in Sofia; **8** initial stages of algal layers of *Apatococcus lobatus*.



Figs 9 - 12:
9, 10 layers of algae and lichens on the granite monument in the museum of D. Debelyanov in Koprivshtitsa with green algae (*Apatococcus* and others) and lichens *Candelariella vitellina* (yellow) and *Lepraria* cf. *neglecta* (whitish); **11** cells of the green algae *Apatococcus lobatus* and *Coccomyxa* spec. (arrow) observed in light microscope immediately after sampling; **12** cells of *Trebouxia arboricola* and *Apatococcus lobatus* (arrows) in light microscope from scraped samples.



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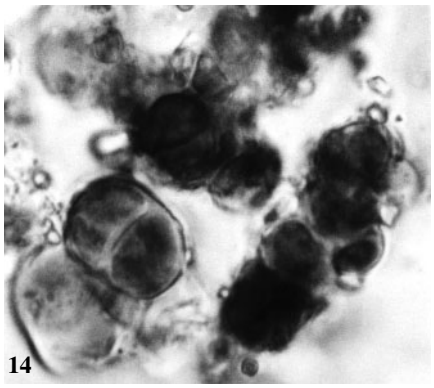

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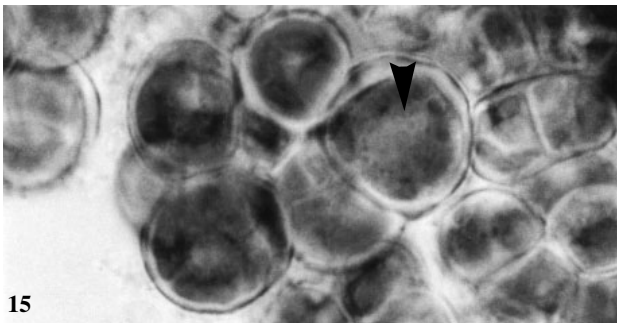

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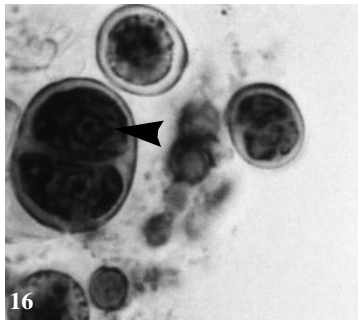

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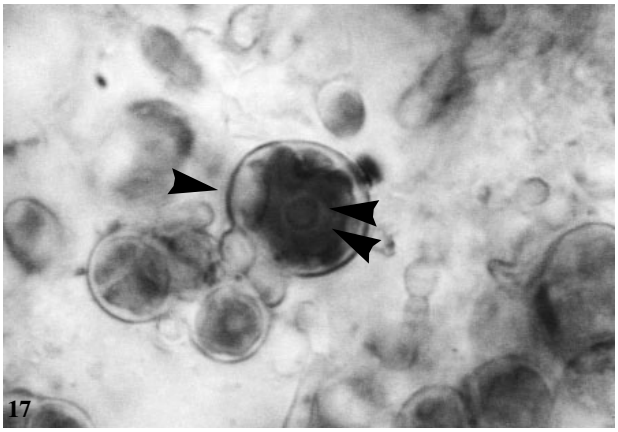

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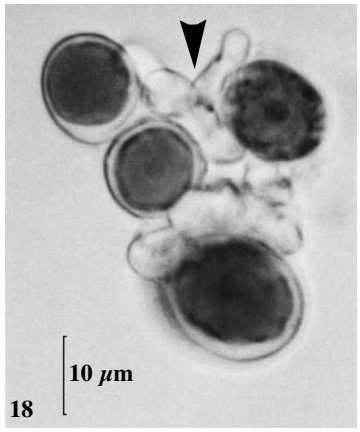

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◄ **Figs 13 - 18:**
aerophytic green algae *Apatococcus* and *Trebouxia* from cultures observed with light microscope; **13** vegetative cells of *Apatococcus lobatus* in diads and sarcinoid packets, grains (lipid droplets?) around the nucleus visible (arrow); **14** cells of *Apatococcus lobatus* with remnants of calcareous material from the monument near the National Art Gallery in Sofia in a non-axenic agar culture after 7 days of cultivation; **15** 4 weeks old cultures of *Apatococcus lobatus* on agar; **16** single cells and diads of *Apatococcus lobatus* in 4 weeks old culture, stained with JKJ, nucleus between lobes of chloroplasts (arrow); **17** vegetative cells of *Trebouxia arboricola* on 4 weeks agar culture, nucleus excentric in a sinus of chloroplast (arrow) and pyrenoid in the centre of chloroplast (double arrowhead), unstained; **18** *Trebouxia arboricola* cells in lichenized stage (fungal hyphae pressed to cell wall, arrow), 4 weeks old culture, unstained.

3. Results and discussion:

The results from this study show that the most important inhabitants of the granite monument in the Koprivshtitsa museum are eukaryotic green algae, forming greenish spots and coloration on the almost whole stone surface in different density. Algal accumulation in a higher density is macroscopically visible in wrinkles of the monument (Figs. 9, 10).

The preliminary analysis with a light microscope of the material from Koprivshtitsa museum showed the presence of 3 algal species with a mass development of *Apatococcus lobatus* (Figs. 11, 13, 15, 16). This alga is well known from tree bark but also occurs on stone, concrete, bricks, wood and other anthropogenic substrates (GEITLER 1942, ETTL & GÄRTNER 1995). *Trebouxia arboricola* (Figs. 12, 17, 18) was also found in the monument material but in lower density. It occurred in a free-living stage but was also lichenized (Fig. 18). The third, but less frequent green alga on the monument surface is *Coccomyxa* (Fig. 11 arrow), which is also known as a common aerophytic and terrestric alga. Single cells of 7-8,5 μm in length and 4-4,5 μm in width, each with one chloroplast without pyrenoid were intermingled within the colonies and layers of *Trebouxia*, as well as of *Apatococcus* (Figs 11, 12). However, without reproduction stages and cultures the determination of this taxon to the species level during this preliminary study was impossible.

The microscopic study of bark samples from the trunk of *Picea abies* nearby the monument showed also dominating *Apatococcus* layers and less frequent cells of *Trebouxia*.

The lichen species occurring on the same monument, but mostly on its backside, were dominated by whitish to greyish spots (up to 4 cm in diameter) of *Lepraria* cf. *neglecta* (NYL.) LETTAU and sterile thalli of *Candelariella vitellina* (HOFFM.) MÜLL.- ARG. (Figs. 9, 10). The same species were observed on the second identic granite monument in the churchyard of Koprivshtitsa. However, there they were restricted to the surrounding stonework and not on the figure surface. Most probably, this is a result of microclimatic dry situation and of the obvious protection from a direct moistening by precipitation due to a granite wall behind the statue. In addition, the lichen species *Protoparmeliopsis muralis* (SCHREB.) M. CHOISY var. *muralis* (syn. *Lecanora muralis* (SCHREB.) RABENH.) and one *Caloplaca* sp. were found there (Fig. 4). All these lichens are widespread in Bulgaria (POPNIKOLOV & ZHELEZOVA1964). Between the lichen thalli particles of green algal layers could be collected which resembled only *Apatococcus lobatus*.

The greenish layers on the calcareous monument in Sofia (Fig. 7, 8) were formed only by developmental stages of *Apatococcus lobatus* (Fig. 8, 14), which doubtless could grow according to the more moistured and overshadowed microclimatic conditions in the backyard of the national art gallery.

The lack of aerophytes on the bronze monument in Sofia (Fig. 6) is doubtless caused by the surface type, by the generally drier climate and by the strong air pollution in this central part of the Bulgarian capital.

In agar cultures *Apatococcus* formed single cells but more frequently characteristic sarcinoid cell packets or sometimes diads and often tetrads, but never short filaments (Figs. 13 - 16). In older cells the parietal lobed chloroplast without a pyrenoid divides in two and grains visible also in light microscope often surround the nucleus (Figs.13, 15 arrows). Morphology and cytology of the studied cells agree well with the results from literature (GÄRTNER & INGOLIĆ 1989, ETTL & GÄRTNER 1995). In cultures *Trebouxia arboricola* cells occurred mostly solitary but also lichenized (Figs. 17, 18), as it was observed during the first microscopic investigations. The cells contain one more or less axial chloroplast, irregularly lobed at the margin, the pyrenoid lacks a distinct starch sheath. The cell's nucleus lies excentric in a distinct sinus of the chloroplast (Fig. 17, arrow). The taxonomic status of *Lepraria* phycobionts is still unknown (WIRTH 1995), but *Trebouxia* as a phycobiont within this genus should not be excluded. Therefore, the detected free-living *Trebouxia* cells could also be originated from phycobionts. There are still doubts that free-living *Trebouxia* taxa occur (AHMADJIAN 2001) but records of some species, for example T. *arboricola* in non-lichenized status from bark and stones exist (BUBRICK et al. 1984, GÄRTNER 1985, MIKHAILYUK et al. 2001). All these records were combined with culture studies. For Bulgaria *Trebouxia arboricola* has been reported from moist tree barks in the Rodopi Mountains, but using of cultures was not mentioned (VODENICHAROV et al. 1971). In the present investigation the occurrence of free-living *Trebouxia arboricola* in Bulgaria is proved by combination of direct microscoping and culture studies.

Apatococcus lobatus was reported many times in Bulgaria under the confusing name *Protococcus viridis* AG. also without mentioning cultural studies (e.g. VODENICHAROV et al. 1971). Therefore, the presented records are the first proven documentation of the occurrence of this species in Bulgaria. The development of *A. lobatus* in the center of Sofia shows again its wellknown resistance against air-pollution and relatively drier air conditions in the towns (BARKMAN 1969).

All the documented aerophyte species are widespread and their occurrence agrees with results from other similar studies. Nevertheless, our knowledge on these organisms is still uncomplete and further investigation of the biodiversity of this ecological group should be done in different regions.

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