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## **Notes on the Akinete-forming Strain of the Green Alga *Klebsormidium dissectum* (*Streptophyta*) from Pirin Mts., Bulgaria**

By

Blagoy Angelov UZUNOV\*), Georg GÄRTNER\*\*), Maya P. STOYNEVA\*)

With 8 Figures

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**Key words:** *Klebsormidium dissectum* (GAY) Ettl & GÄRTNER, *Klebsormidio-phyceae*, *Streptophyta*. – Akinete formation, green algae, soil algae. – Flora of Pirin Mts., Bulgaria.

### Summary

UZUNOV B. A., GÄRTNER G. & STOYNEVA M. P. 2012. Notes on the akinete-forming strain of the green alga *Klebsormidium dissectum* (*Streptophyta*) from Pirin Mts., Bulgaria. – *Phyton* (Horn, Austria) 52 (1): 139–144, with 8 figures.

From the Pirin Mts. National Park in Bulgaria soil samples were investigated for the content of algae. Among them, three species of the streptophyte genus *Klebsormidium* SILVA, MATTOX & BLACKW. were identified. One strain of *Klebsormidium dissectum* (GAY) Ettl & GÄRTNER with akinete formation was observed. The term “akinete” (“hypnoblast”) is used because of the inclusion of the original cell wall of the former vegetative cell in the wall of the resting stage. Since the presence of akinetes has been rarely observed in *Klebsormidium* (or just rarely documented and reported) in the taxonomic literature, their finding is interesting as a documented confirmation of this mode of reproductive- and resting cell formation. Therefore, in the present work, the most characteristic morphological and reproductive features of the studied isolate are presented.

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\*) Assist. Dr. Blagoy Angelov UZUNOV and Prof. Dr. Maya P. STOYNEVA, Department of Botany, Faculty of Biology, Sofia University “St Kliment Ohridski”, 8 bld. Dragan Zankov, 1164-Sofia, Bulgaria; e-mail: blagoy\_uzunov@abv.bg; mstoyneva@abv.bg

\*\*) Univ.-Doz. Dr. Georg GÄRTNER (corresponding author), Institut für Botanik der Universität Innsbruck, Sternwartestrasse 15, 6020 Innsbruck, Austria; e-mail: georg.gaertner@uibk.ac.at

## Zusammenfassung

UZUNOV B. A., GÄRTNER G. & STOYNEVA M. P. 2012. Notes on the akinete-forming strain of the green alga *Klebsormidium dissectum* (*Streptophyta*) from Pirin Mts., Bulgaria. [Bemerkungen zu einem Akineten-bildenden Isolat der Grünalge *Klebsormidium dissectum* (*Streptophyta*) aus dem Pirin-Gebirge (Bulgarien)]. – *Phyton* (Horn, Austria) 52 (1): 139–144, mit 8 Abbildungen.

Im Pirin-Nationalpark in Bulgarien wurden Bodenproben gesammelt und auf den Gehalt an Algen untersucht. Darunter fanden sich drei Arten der Streptophytengattung *Klebsormidium* SILVA, MATTOX & BLACKW. Ein Stamm von *Klebsormidium dissectum* (GAY) Ettl & GÄRTNER bildete Akineten. Wegen des Einschlusses der Original-Zellwand der vegetativen Zelle in die Wand der Dauerzelle wird der Terminus „Akinet“ („Hypnoblast“) verwendet. Da das Vorhandensein von Akineten in der taxonomischen Literatur bei *Klebsormidium* selten beobachtet oder zumindest dokumentiert wurde, sind die vorgelegten Beobachtungen eine Bestätigung dieser Form der Dauerzellen-Bildung. Die morphologischen Charakteristika dieses Fortpflanzungsmodus im studierten Isolat werden dargestellt.

## 1. Introduction

In the last years soil and aerophytic algae from the Pirin Mts. – National Park and UNESCO Monument of Natural Heritage in Bulgaria – were investigated and cultivated. About 130 strains of cyanoprokaryotes, chlorophytes, ochrophytes and streptophytes were isolated, see for e. g., UZUNOV 2009, UZUNOV & al. 2008 and GÄRTNER & al. 2010. Three species of the streptophyte genus *Klebsormidium* SILVA, MATTOX & BLACKWELL (*Klebsormidiales*, *Klebsormidiophyceae*) were identified: *K. flaccidum* (KÜTZ.) SILVA, MATTOX & BLACKWELL, *K. montanum* (SKUJA) S. WATANABE and *K. dissectum* (GAY) Ettl & GÄRTNER. One strain of *K. dissectum* with akinete formation was observed. Up to now the presence of akinetes in *Klebsormidium* has been rarely reported in the taxonomic literature. The terminology of reproductive and resting cells/stages is still unclear. The confusion comes from the fact that most of the terms are taken in a very broad sense and are used with different meanings by different authors, even in the most modern phycological literature (GRAHAM & al. 2009, LEE 2008, etc.). Therefore in the present work, the most characteristic features of the studied isolate are shown and some terms are discussed.

## 2. Material and Methods

The study is based on alpine soil samples collected in August 2006 from 13 sites in the Pirin Mts. (see details in UZUNOV & al. 2008). In the laboratory, from each sample a liquid soil solution was prepared. The algal cells from these solutions were inoculated onto agar plates by using the atomized cell spray technique (PRINGSHEIM 1946, ANDERSEN 2005). Axenic clonal cultures were obtained and kept in sterile agar tubes with Bold's Basal Medium (BBM; BISCHOFF & BOLD 1963). The akinete-forming *Klebsormidium dissectum* was isolated from sample nr. 11 (near Lake Bezbog) collected at 2249 m a.s.l. and 10 cm below the soil surface. The samples and cultures are

deposited in the Algal Collection of the Department of Botany of Sofia University 'St Kliment Ohridski' (ACUS). Cultures are maintained at room temperature (21°C) with an irradiance of ~60  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  and a light:dark regime of 14:10 h.

Microscopic investigations were made using a Diapan Microscope Reichert with objectives 10 $\times$ , 25 $\times$ , 63 $\times$  and 100 $\times$ . Photomicrographs were taken with a Moticam 2000 camera and software 'Motic Images Plus 2.0' was used.

The terminology used follows Ettl 1980, 1983 and 1988, Ettl & Gärtner 1995 and Temníšková & Stoyneva 2011.

### 3. Results and Discussion

The green algal genus *Klebsormidium* was formerly known under the names *Hormidium* Kütz. and *Chlorhormidium* Fott (Printz 1964, Starmach 1972, Ettl & Gärtner 1995, Hindák 1996, Lokhorst 1996, Škaloud 2006). *Klebsormidium* contains around 15 infrageneric taxa and is accepted as an important member of *Klebsormidiales* (Sluiman & al. 2008) of *Charophyceae*, which is supposed to be close to the ancestor of the land plants (Lokhorst 1996, Novis & al. 2008, Graham & al. 2009).

About ten species of this genus play an important role in aero-terrestrial ecosystems (Lokhorst 1996). One of the most common among them is *Klebsormidium dissectum* (Gay) Ettl & Gärtner 1995 [Synonyms: *Stichococcus dissectus* Gay 1891, *Hormococcus flaccidus* var. *dissectus* (Gay) Chodat 1902, *Hormidium dissectum* (Gay) Chodat 1913, *Chlorhormidium dissectum* (Gay) Farooqui 1969].

According to the descriptions, this alga appears in cultures in straight or slightly bent unbranched filaments, formed by cylindrical or slightly barrel-shaped cells. They easily dissect into short filaments, few-celled (2–4) fragments or even into single cells (for details see Printz 1964, Starmach 1972, Ettl & Gärtner 1995, Hindák 1996, Lokhorst 1996). Asexual reproduction in cultures runs mainly by sporulation, in which motile and non-motile spores are formed (planospores = zoospores and aplanospores, respectively). The second way of reproduction, which offers a possibility to survive harsh conditions, is the formation of non-motile resting cells, which are commonly named akinetes.

In our material the vegetative cells were 5.5–8.0  $\mu\text{m}$  wide and 9.5–14  $\mu\text{m}$  long, cylindrical to barrel-shaped. Each cell contained one plate-shaped chloroplast with one pyrenoid surrounded by starch grains. In old and drying cultures some vegetative cells within the filaments or at their end became obviously larger than the other cells. These intercalary or terminal cells were 18–20  $\mu\text{m}$  in diameter, globular, ellipsoidal or irregular in shape and it was possible to see them even by a hand-lens with 10 $\times$  magnification. Within the original cell wall secondary and tertiary wall layers were formed, they appeared in outline as lamellate or sometimes sculptured (Fig. 1, 2, 5–7). Afterwards, these large and thick-walled cells were released from the filaments by fragmentation into groups (Fig. 4, 8) or sin-

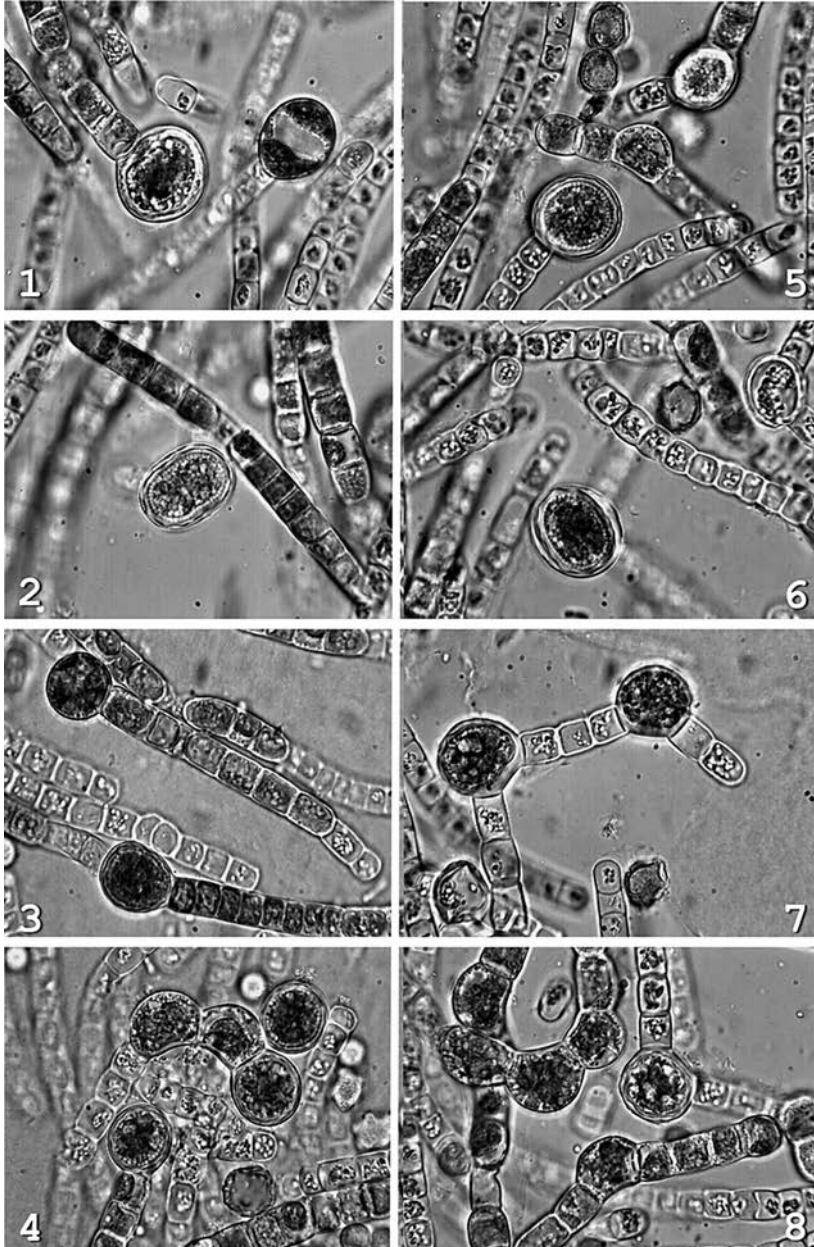


Fig. 1–8. Different stages of akinete (“hypnoblast”) development in *Klebsormidium dissectum* (GAY) Ettl & GÄRTNER. – Fig. 1, 4 terminal akinetes. – Fig. 3, 5, 7 intercalar akinetes. – Fig. 4, 8 groups of akinetes released from filaments. – Fig. 2, 6 lamellated and sculptured akinetes in different stages of development.

gularly (Fig. 1, 3, 5, 7). In four months old cultures only solitary large, thick-walled cells were found resembling typical akinetes *sensu auct.* (Fig. 2, 6).

Commonly, akinetes have a main function as resting stages under unfavourable environmental conditions and therefore they are also named resting spores. Another term for resting spores is “Dauerzellen” according to PASCHER 1927, who defines them as a type of non-motile spores or aplanospores (for more details see Ettl 1980, 1983). Later on, Ettl 1988 enlarged his discussions from 1980 and 1983 and proposed a more detailed terminological splitting for reproductive and resting stages of algae, based on their cytological peculiarities, providing supporting illustrations. His terminology was accepted and explained again in the manuals by Ettl & Gärtner 1995 and Temnísková & Stoyneva 2011 and was applied in the taxonomic works of Hindak 1996 and Lokhorst 1996. Following the definition of Ettl 1988, every reproductive sporulation type and therefore also the aplanospores produce totally new cell walls in their daughter products (after a previous protoplast division) without involving the original cell wall in their formation. According to the same author, when these reproductive cells function as resting stages to survive harsh conditions, they have to be named “hypnospores”. By contrast, the term “hypnoblast”, or its synonym “akinete” *sensu auct.*, is defined by Ettl 1988 as a resting stage formed directly from a vegetative cell where the original cell wall is included in the formation of the new thick and layered protective wall. Taking into account the observations on the strain from Pirin Mts. material, we propose to use for the resting cells/stages of *Klebsormidium dissectum* only the term “akinete” or “hypnoblast” in the sense of Ettl 1983, 1988 and Ettl & Gärtner 1995 (Fig. 1–8), but not “aplanospore” or “hypnospore”.

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Artikel/Article: [Notes on the Akinete-forming Strain of the Green Alga Klebsormidium dissectum \(Streptophyta\) from Pirin Mts., Bulgaria 139-144](#)