# Cyanocystopsis kitagatae gen. et sp. nov. (Cyanoprokaryota/ Cyanobacteria) from the tropical lake Kitagata (Uganda, Africa)

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# *Cyanocystopsis kitagatae* gen. et sp. nov. (Cyanoprokaryota/ Cyanobacteria) from the tropical lake Kitagata (Uganda, Africa)

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Jean-Pierre Descy & William Okello

*Summary:* The paper describes a new genus and new species of Cyanoprokaryota, referred to Pleurocapsales incertae sedis – *Cyanocystopsis kitagatae* gen. et sp. nov. The new taxon is characterized by the presence of two different stages (filamentous, formed by spherical cells and stalked bundles of claviform cells). The branching of filaments, which leads to the formation of clusters of claviform cells, is caused by cell division, which slightly resembles the true-branching of cyanoprokaryotes. However, this division is peculiar by its subsequent character and excentric disposition of the daughter cells, which leads to the formation of specific tetrads, from which the claviform cells develop. Spores (aplanospores) and vegetative reproductive stages of the alga have been observed. The new species was found as a dominant in a fixed phytoplankton sample from the small, hypertrophic and hypersaline tropical crater lake Kitagata (Uganda, Africa). The pigment marker analysis of the same sample proved the high (ca 96%) cyanoprokaryote contribution to the phytoplankton biomass.

*Keywords:* algal reproduction, cell division, crater lake, hypersaline lake, hypertrophic lake, phytoplankton, Pleurocapsales, shallow lake

Cyanoprokaryota/Cyanobacteria are one of the most challenging groups for recent classification (Komárek et al. 2014). This is especially valid for the representatives of the order Pleurocapsales, the taxonomic position and category of which has been changed several times after its first proposal by Geitler (1925) (for details see Komárek et al. 2014). This order contains interesting species from genera *Cyanocystis*, *Dermocarpa*, *Dermocarpella* and *Chamaecalyx*, which develop as attached to different substrata (being mainly epiphytic on filamentous algae) or as endophytic in *Sphagnum* mosses and form characteristic groups of claviform or pear-like sporangia. However, the vegetative stages of these species are almost unknown (Geitler 1932). In the present paper (following the International Code of Nomenclature for algae, fungi, and plants ICNAFP, Turland et al. 2018), we describe an alga with a filamentous vegetative stage, which forms bundles of claviform cells (most probably functioning mainly as sporangia). It was found as free-living in both stages in a planktonic sample collected in the small African lake Kitagata. Due to the fixed character of the sample, the cultivation of the species with documenting the sporulation process and molecular-genetic analysis could not be realised, but the clear, outstanding unique morphological features and observed reproduction stages allow us to suppose that it represents a new species, which belongs to a new genus. The peculiar two-stepped, successive cell division with first specific excentric disposition of daughter cells in triads, which leads to the formation of cell tetrads and then clusters of claviform cells, according to our knowledge, has not been described for cyanoprokaryotes so far. The new genus resembles *Cyanocystis* at first glimpse, and therefore it is named *Cyanocystopsis.* It could be tentatively supposed that this new genus represents a new family and even a new order of Cyanoprokaryota. But for now, due to lack of

the molecular-genetic data, according to the unique cyto-morphological features, we place it in Pleurocapsales incertae sedis.

## Materials and methods

The material was collected on 8.04.2017 from the small tropical crater lake Kitagata (Uganda, Africa). The lake was sampled once during the HIPE project, supported by the Belgian Science Policy Office (BELSPO, contract # BR/154/A1/HIPE), but it had been studied before by Russell et al. (2007) for climate reconstruction in Africa and by Ma et al. (2011) for its peculiar geochemical features, involving brine formation. According to Ma et al. (2011), lake Kitagata is a small (0.62 $km^2$), perennial saline lake located in the western branch of the East African Rift System, southwestern Uganda, lying within a 1.8 $km^2$ steep-walled crater (Fig. 1).

According to the HIPE project data, the lake is around 5 m deep (up to 9 m according to Ma et al. (2011)), has strongly alkaline (pH 9.81; total alkalinity 891 mmol $kg^{-1}$) and hypersaline (conductivity 0.138 S $m^{-1}$) waters and is hypertrophic (chlorophyll *a* 1170 µg $L^{-1}$).

A 250 ml surface sample was collected by the HIPE team and preserved with formaline (1% final concentration) in the field and later sedimented to a volume of 25 ml. It was subsequently processed on the light microscope (LM) Motic B1. The microphotographs were taken by a Moticam 2.0 mp camera supplied by Motic Images 2 Plus software program. Twenty five non-permanent slides were observed under magnification 40× and under immersion oil at magnification 100×. Determination followed standard taxonomic keys on Cyanoprokaryota

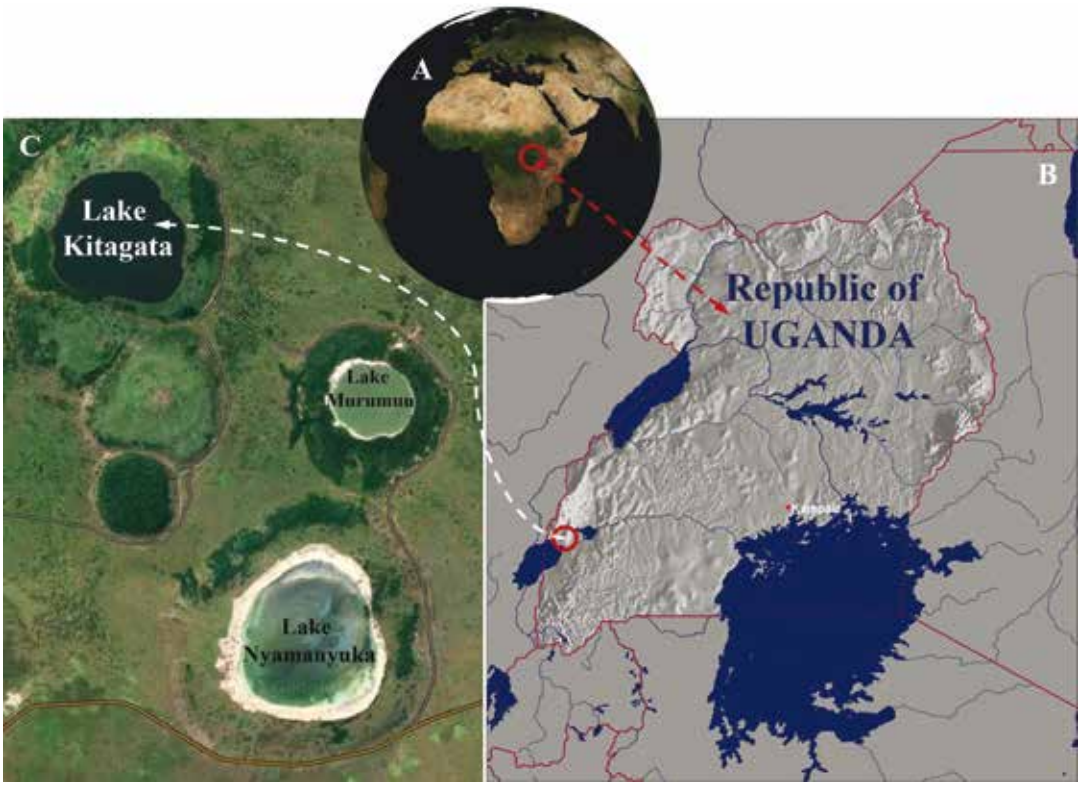


**Figure 1.** Map of Africa (A) and of the Republic of Uganda (B) with indication of the crater lake Kitagata (C) (modified after Ginkgo Maps and Google Maps).

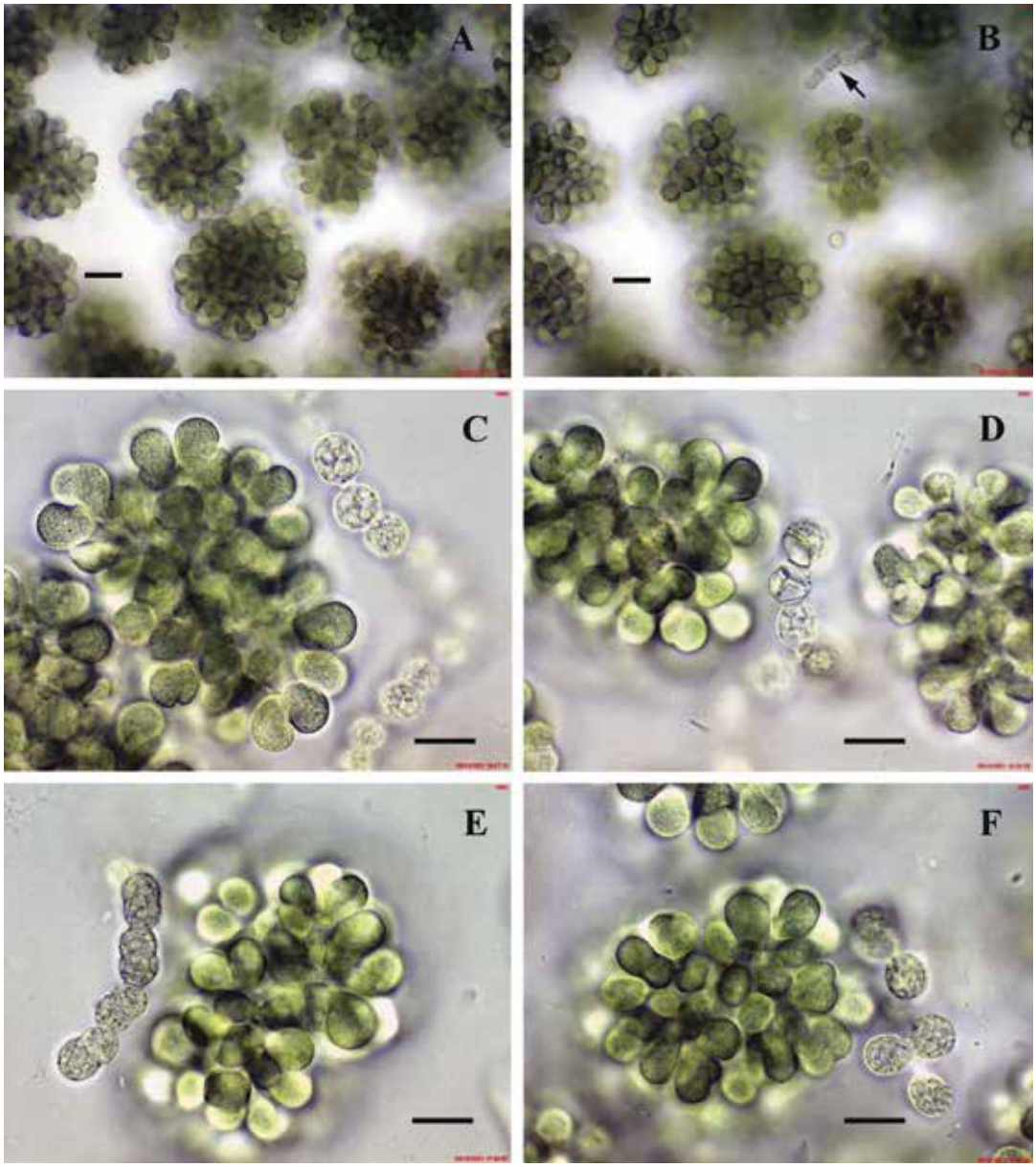


**Figure 2.** *Cyanocystopsis kitagatae* gen. et sp. nov. in the phytoplankton sample from lake Kitagata at low (40×) LM magnification (A–B) and high (100×) LM magnification under immersion (C–F). Clusters of claviform cells (A); Uniseriate filament (arrow) among the abundant clusters of claviform cells (B); Cluster of claviform cells and uniseriate filamentous stage with spherical cells with net-like content (C); Clusters of claviform cells and filamentous stage at initial stage of branching (D); Cluster of claviform cells and uniseriate filamentous stage with elongated cells in a process of binary division (E); Cluster of claviform cells and uniseriate filamentous stage with spherical cells in a process of ramification and formation of an initial tetrad (F). Scale bars = 20 µm (A, B) and 10 µm (C–F).

(Geitler 1925, 1932, 1942; Elenkin 1936–1949; Gollerbakh et al. 1953; Starmach 1966; Komárek & Anagnostidis 2008).

During the project, marker pigment analysis was carried out on a surface water sample, filtered on a glass-fiber filter (Macherey-Nagel GF5, nominal pore size 0.7 mm) under a mild vacuum. Phytoplankton pigments were extracted in 90% HPLC-grade acetone and analysed using high

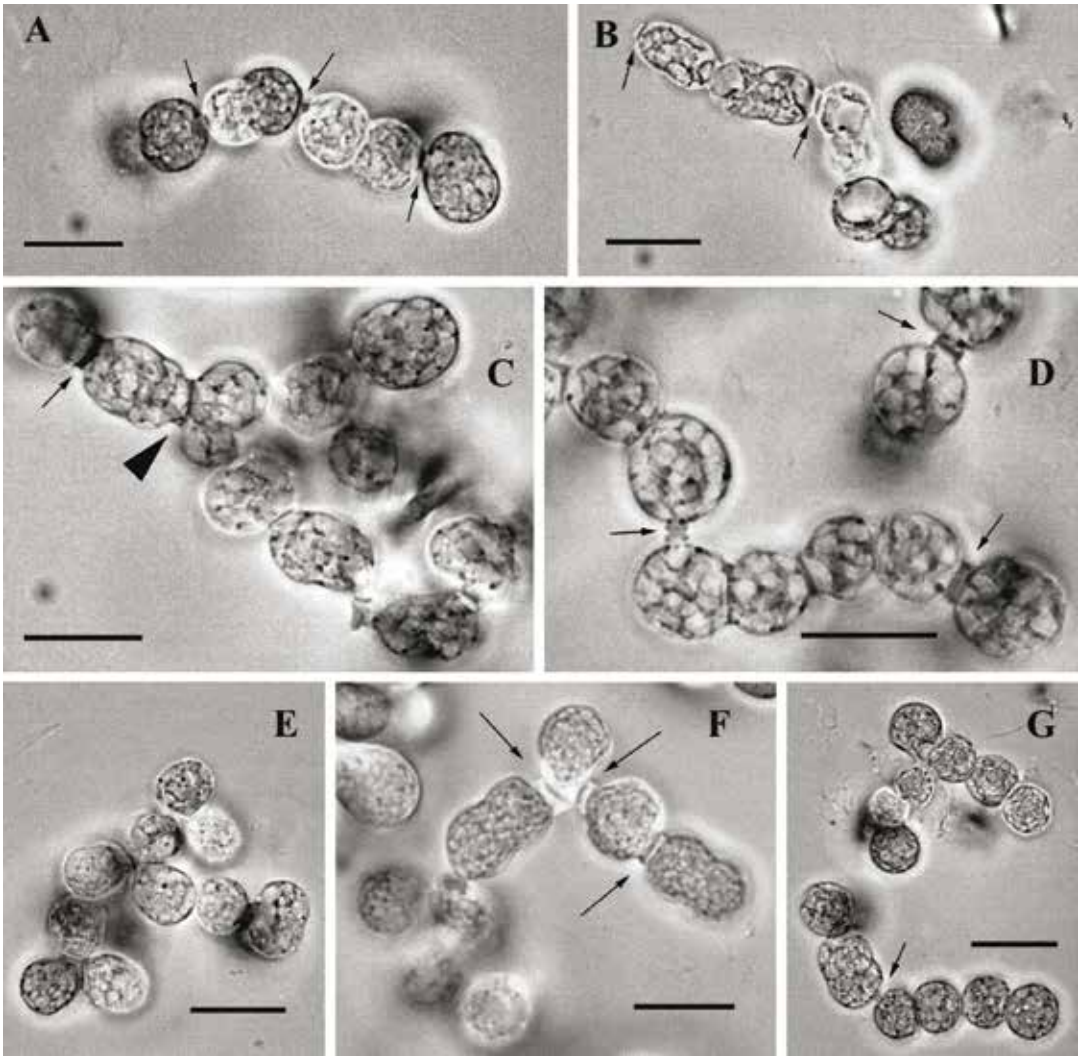


**Figure 3.** *Cyanocystopsis kitagatae* gen. et sp. nov. Filamentous stage with cells with net-like content under high (100×) LM magnification and immersion. Uniseriate filament with spherical cells with visible thick short connections between the cells (A); Uniseriate filamentous stage with elongated cells in a process of binary division (B); Filamentous stage in initial stage of branching with specific division, which leads to the formation of triads (C); Uniseriate filaments with spherical cells with visible bipartite connections between the cells (D); Branching of filament (E); Initial branching with well visible thick short cell connections (F); Filaments with cells at different division stages with visible elongated connection between the cells (G). Thin arrows point at the peculiar cell connections (A–D, F, G), arrowhead points at the specific division, which leads to the formation of a triad (G). Scale bars = 10 µm.

performance liquid chromatography (HPLC), as described in Sarmento et al. (2006). Pigment concentrations were processed using Chemtax (Mackey et al. 1996) in order to determine phytoplankton composition and biomass according to Stoyneva-Gärtner et al. (2020).

## Results

### Results from observations by light microscopy and marker pigment analysis

According to the HPLC analysis of marker pigments, the phytoplankton extracts contained essentially cyanoprokaryote pigments (e.g. aphanizophyll, myxoxanthophyll), along with lower

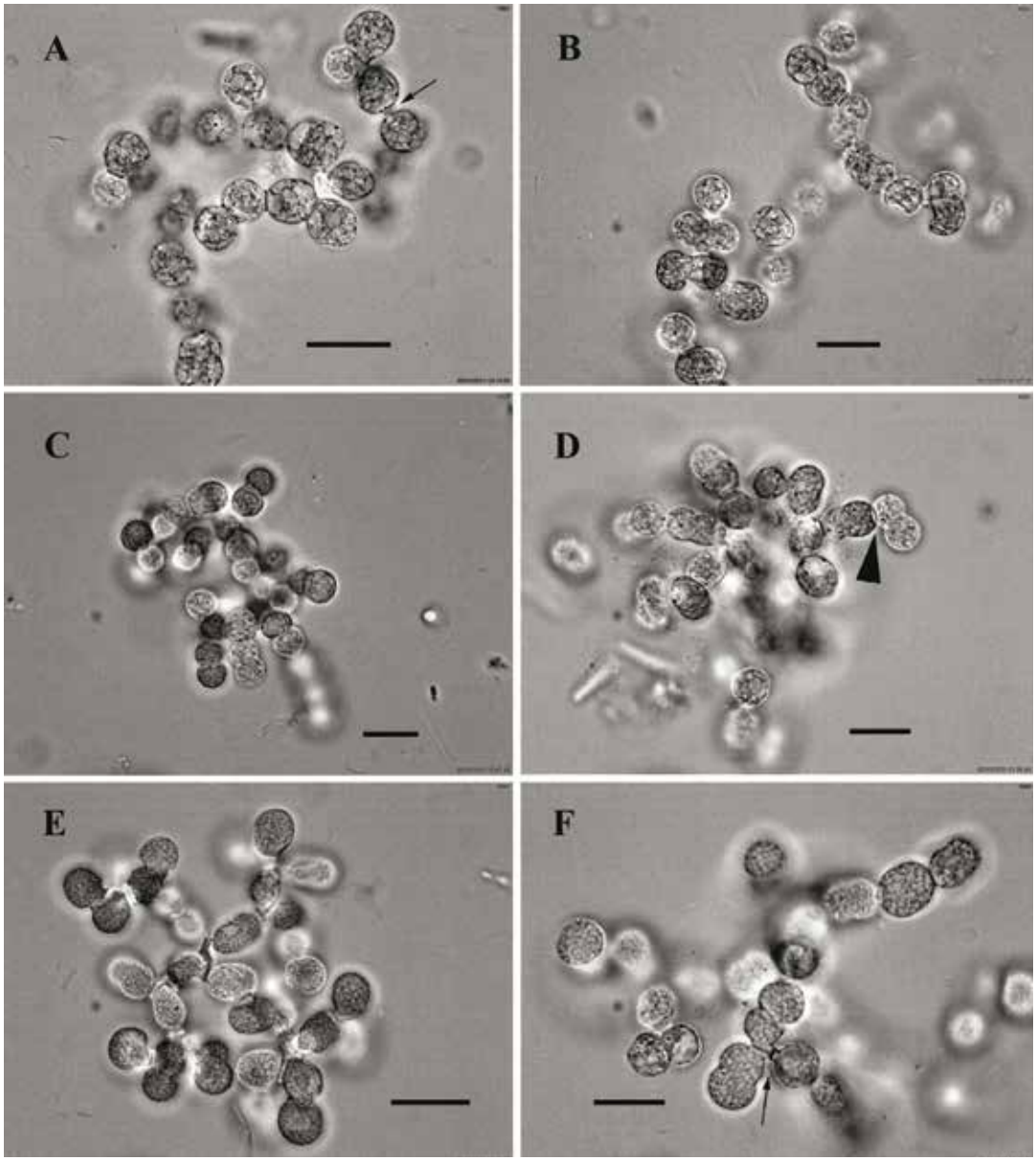


**Figure 4.** *Cyanocystopsis kitagatae* gen. et sp. nov. Filaments of cells with net-like content in different stages of development and division stages under high (100×) LM magnification and immersion (A–F). Multiple branching of filaments (A–B); Filaments with transversal divisions of spherical cells resembling the process of true branching (C); Filaments with transversal divisions of spherical cells resembling the process of true branching and peculiar division to a triad (D); Filaments with numerous initial tetrads (E–F). Thin arrows point at the specific cell connections between the cells with net-like content (A, F), arrowhead points at the specific division, which leads to the formation of a triad (D). Detailed explanation in the text. Scale bars = 10 µm.

concentrations of antheraxanthin and alloxanthin, attributed respectively to Chrysophyceae and Cryptophyta. According to the estimates from the Chemtax runs, cyanoprokaryotes formed ca 96% of the phytoplankton biomass.

The same result on cyanoprokaryote dominance was obtained by conventional LM, when dense populations of free-flowing bright blue-green colonies, resembling *Gomphosphaeria* or *Woronichinia*

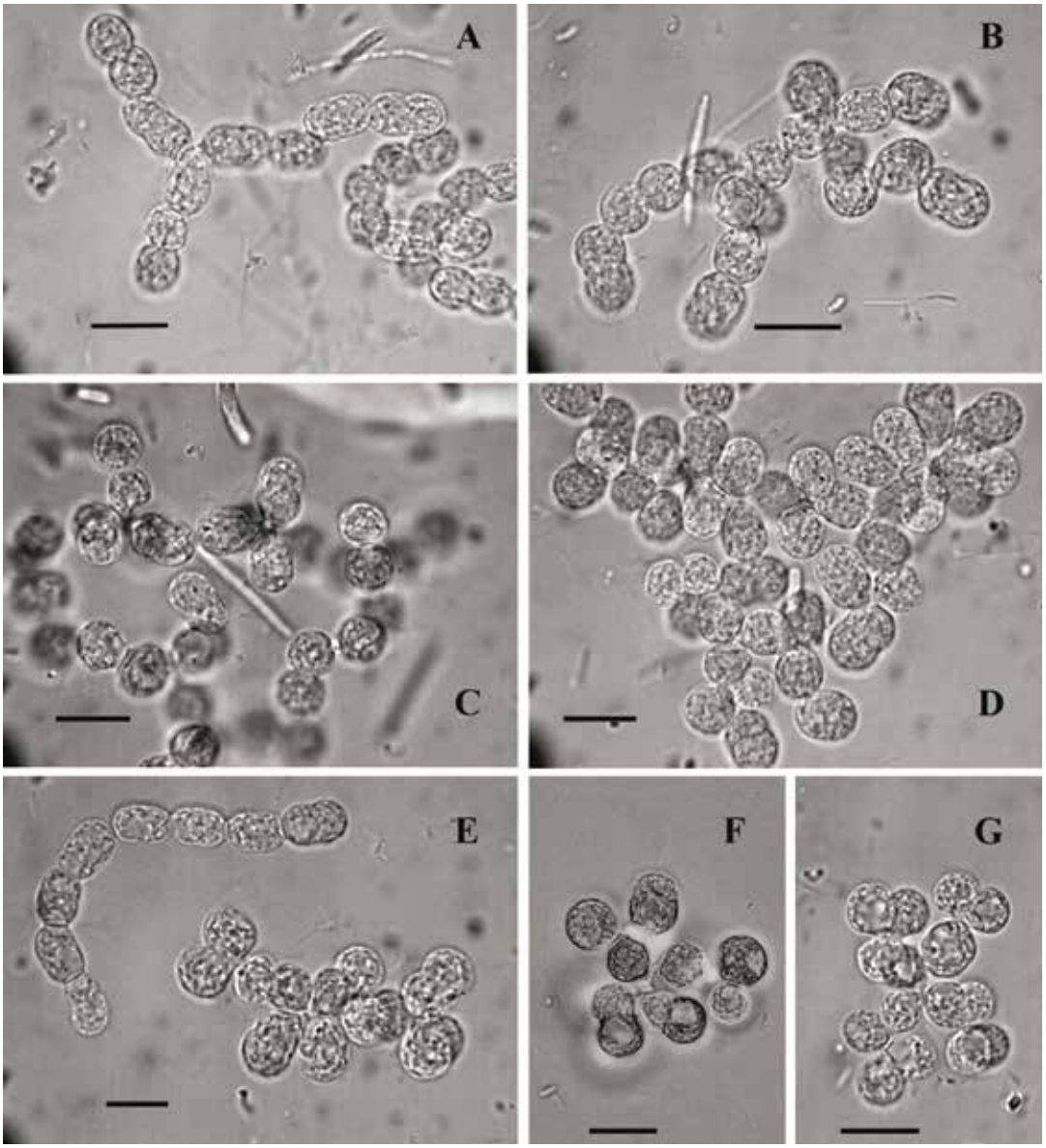


**Figure 5.** *Cyanocystopsis kitagatae* gen. et sp. nov. Filaments of cells with net-like content in different stages of development till formation of tetrads under high (100×) LM magnification and immersion (A–G). Detailed explanation in the text. Scale bars = 10 µm.

groups, were observed at low magnifications (Fig. 2A). However, further investigations revealed the presence of uniseriate filaments (Figs 2B, C) and all stages of the development of these filaments till formation of complex clusters of claviform cells (Figs 2D–F, 2–9).

Under immersion, it was seen that all cells in the uniseriate filaments had a visible net-like (?keritomic) content (Figs 2C–F, 3–5; 8G; 10B). Cells are spherical, up to 6–6.5 µm in diameter (Figs 2C, D; 3A, D, E, G; 9B), but during the transversal cell division, they elongate, reaching 10–12 µm in length (Figs 2E; 3B, C, F, G; 4F; 5A, E; 8G; 10B). Very peculiar are the thick connections between the cell walls (Figs 3B–G; 4A, F; 8G), which are visibly formed by two parts (Fig. 3D).

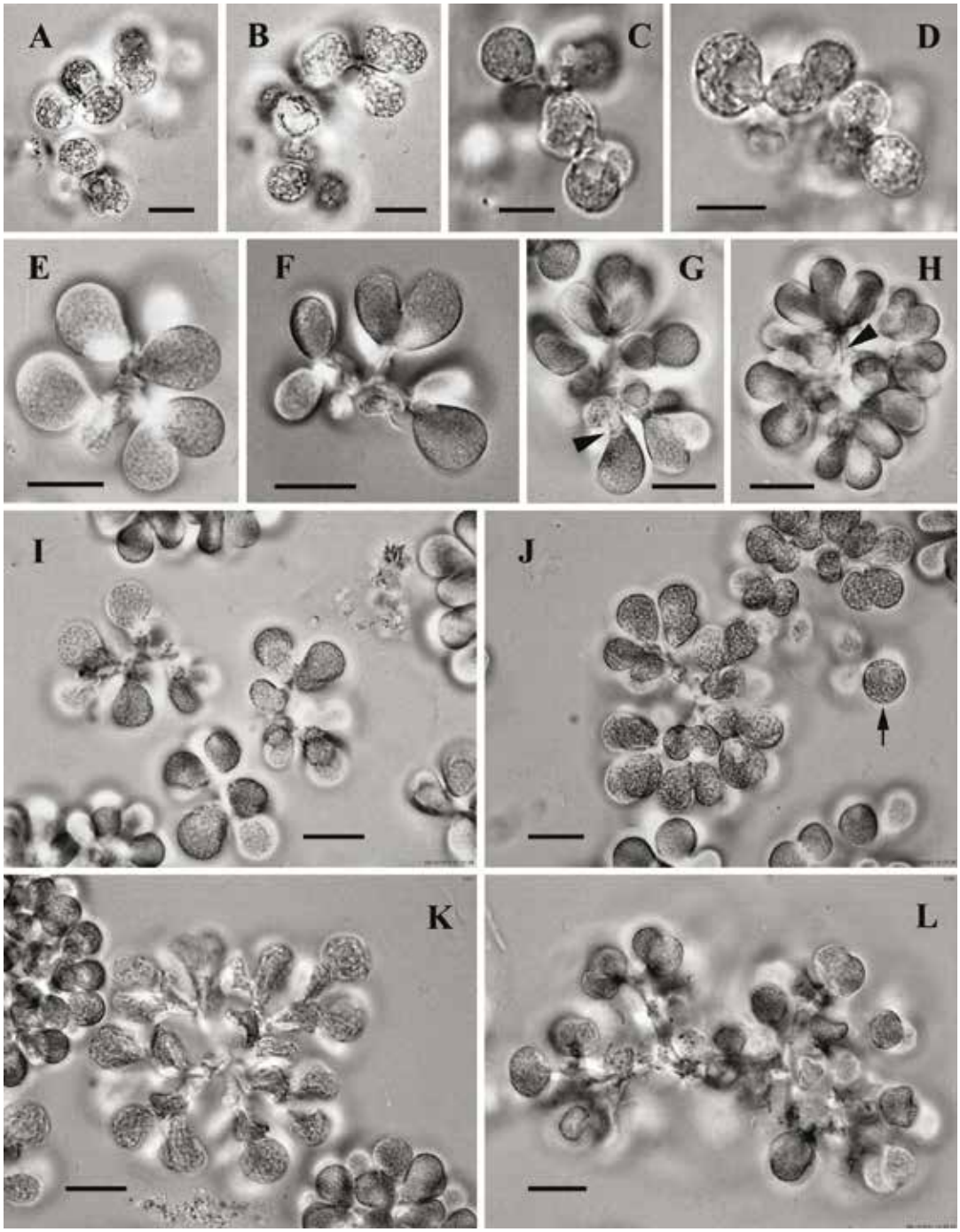


**Figure 6.** *Cyanocystopsis kitagatae* gen. et sp. nov. Clusters in different stages of development and visible cell connections and stalks under high (100×) LM magnification and immersion (A–L). Detailed explanation in the text. Arrowheads point at stalks (G, H), thin short arrow points at the top view of a claviform cell (J). Scale bars = 10 µm.

Branching of uniseriate filaments starts by cell division in a transversal plane (Figs 2F; 3C, E–G) and, by time, becomes more frequent (Figs 4; 5B–E; 10B). Transversal divisions can run in several neighbouring cells at the same time, first resembling the process which leads to the true-branching in cyanoprokaryotes (Fig. 4C, D). However, the peculiarity is in the further disposition of the

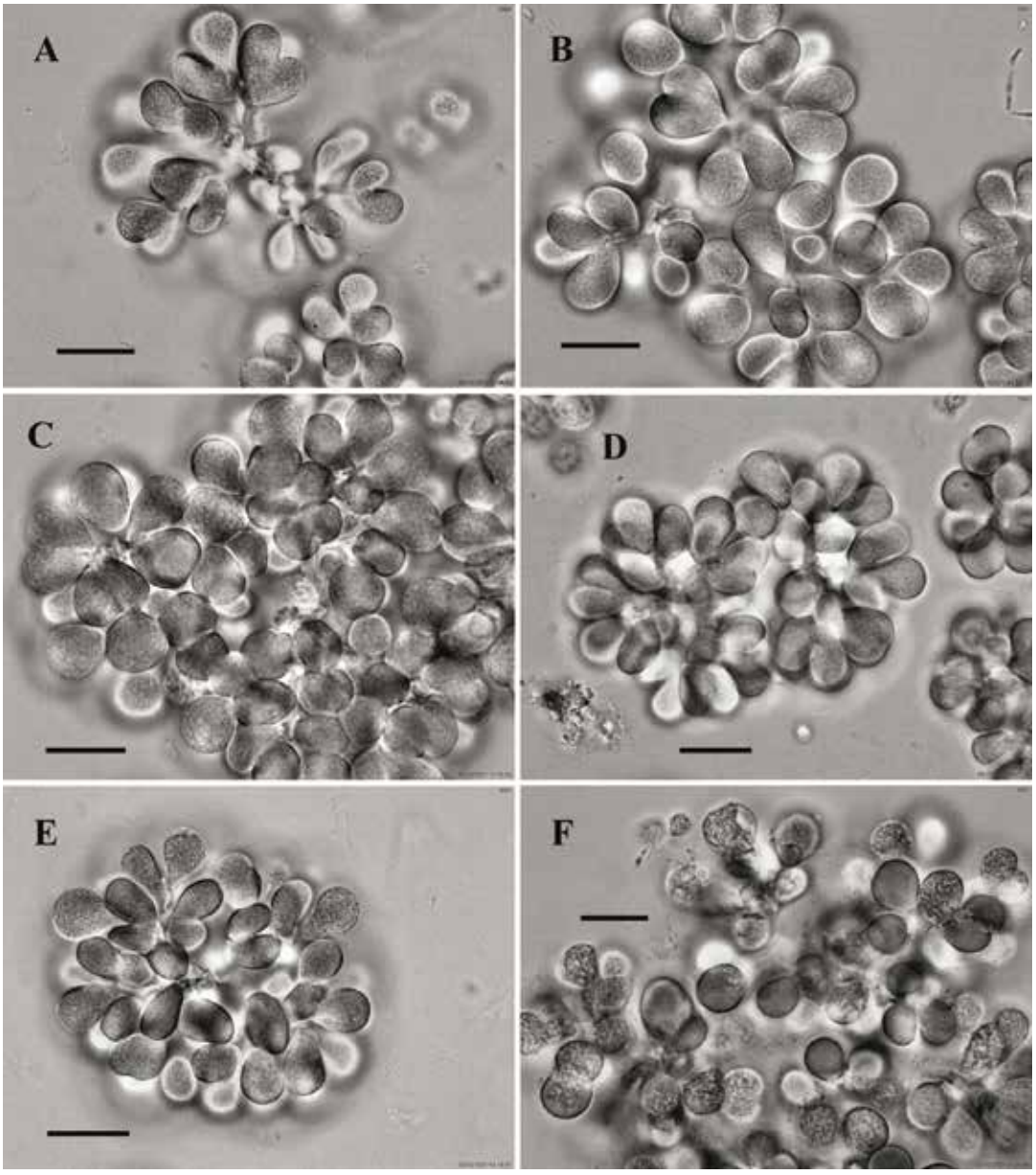


**Figure 7.** *Cyanocystopsis kitagatae* gen. et sp. nov. Clusters in different stages of development under high (100×) LM magnification and immersion: Clusters with homogenous content of cells (A–D); Clusters with cells in different stages with homogenous and net-like content (E–F). Detailed explanation in the text. Scale bars = 10 µm.

daughter cells and development of triads and then, after one more division of tetrads, in which at least one of the cells lies in a different plane (Figs 3C, E–G; 4A, B, D–F, 5A–E). Intensive divisions of the cells in branched filaments form numerous tetrads (Figs 4E; 5C, D). Each cell of these initial tetrads can further divide (Figs 5E–G; 6A–D). During the whole process of tetrad formation, the cell content remains net-like (Figs 3–6A–D).

After formations of tetrads, cells start to elongate, reaching up to 10–(12) µm in length and become claviform with visible stalks (Fig. 6E–L). It seems that the cell content of the claviform cell is reorganised in a way that its lower part firstly becomes transparent and then separates as a

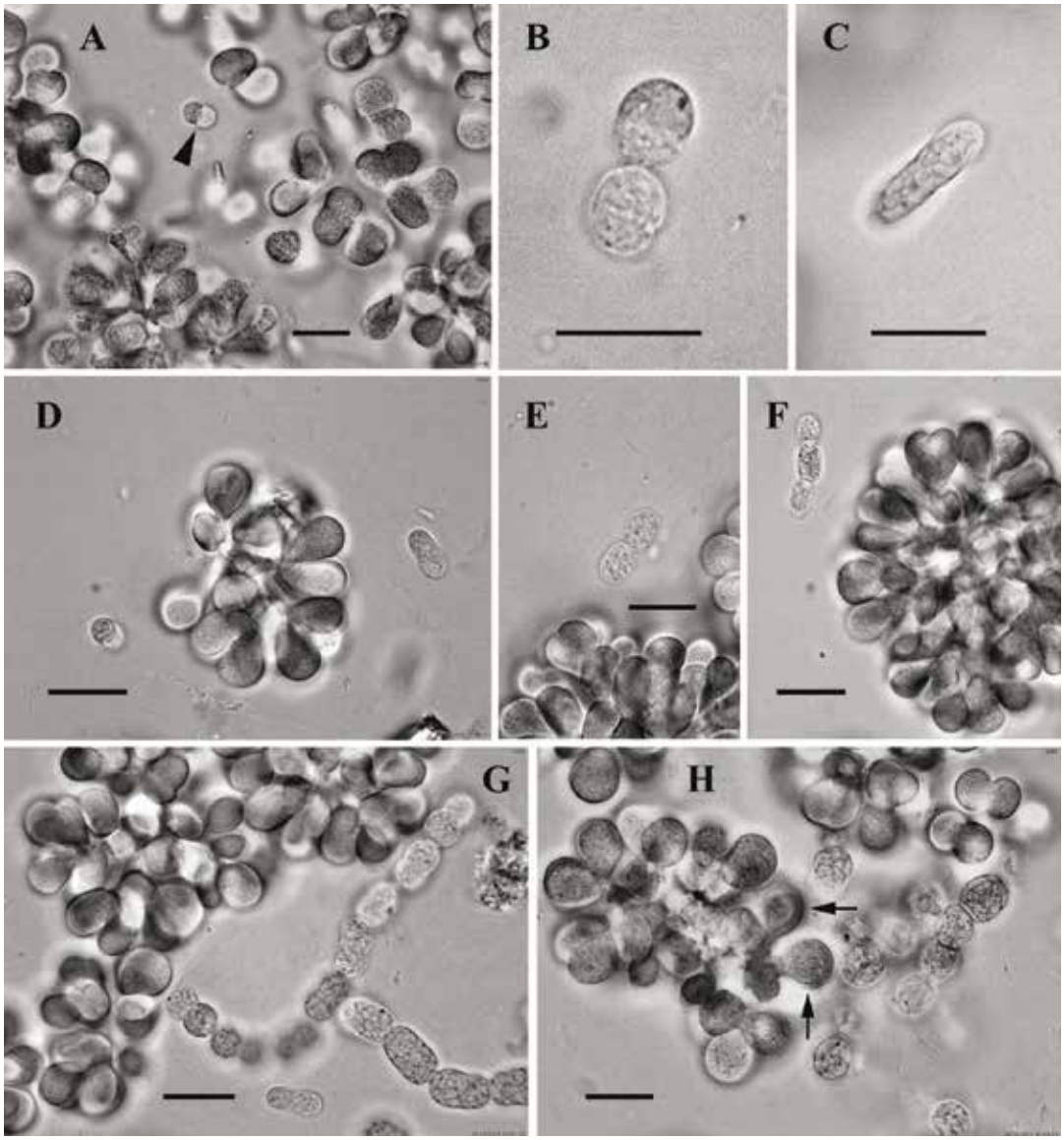


**Figure 8.** *Cyanocystopsis kitagatae* gen. et sp. nov. Spores in different stages of development till formation of filaments with net-like content under high (100×) LM magnification and immersion. Single spore in a process of division among the clusters of claviform cells (A); Growing spores with net-like content in a process of division (B, C); Dividing spores with net-like content near to clusters of claviform cells (D–F); Single dividing spore and filament, which contains both spherical and elongated dividing cells with net-like content (G); Cluster with claviform cells and two peculiar structures with larger upper cell and supporting thick short cell, which possibly would develop as sporangia (H). Arrowhead points at the spore (A) and thin arrows point at both peculiar two-celled structures (H). Scale bars = 10 µm.

stalk (Fig. 6G). Sometimes, the basic connection of the stalks is well visible (Fig. 6E), strongly resembling the two-partite connections between divided cells. Claviform cells of the cluster in the beginning have the same net-like structure as the cells of the filamentous stage (Fig. 6I–L). By time, their cell content becomes homogenous (Figs 2A–F; 7A–D; 8D–G; 9A–B; 10A). Cells look spherical from the top (Fig. 6J). The claviform cells divide longitudinally in a vertical plane (Figs 6E–L; 7A–F). Obviously, multiple longitudinal divisions lead to the formation of more and more dense clusters of stalked claviform cells, which reach ca 45–85–100–125 µm in diameter

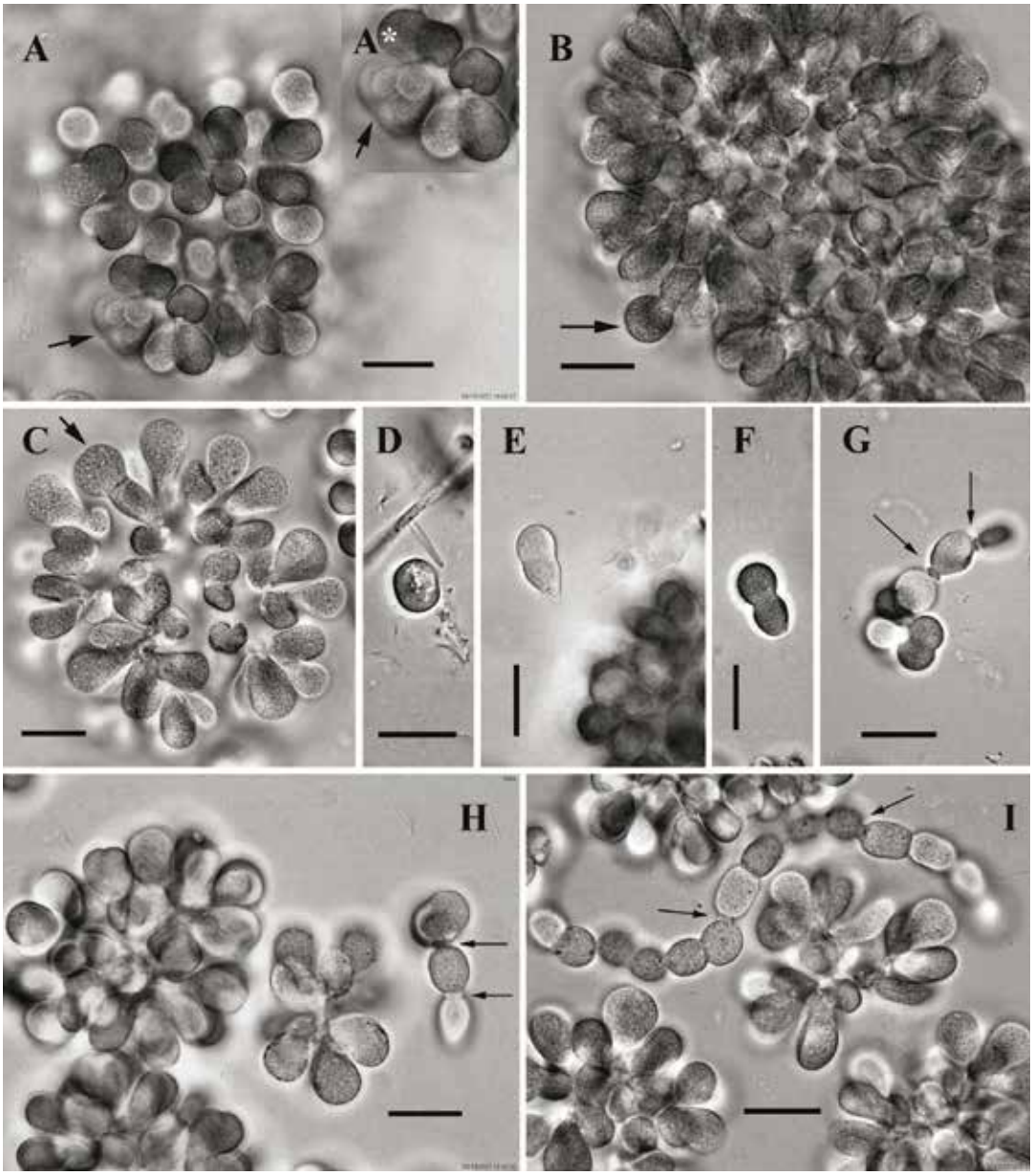


**Figure 9.** *Cyanocystopsis kitagatae* gen. et sp. nov. Different stages of specific vegetative reproduction under high (100×) LM magnification and immersion. Cluster with a single peculiar cell supposed to be a sporangium (A) and the same cell enlarged to show the common cell wall and smaller cells inside (A*); Different clusters, each with a single spherical cell, formed after transversal division of a claviform cell (B, C); Detached spherical cell, supposed to be an initial gemma and its division to uniseriate filaments of cells with homogenous content (D–I). Short arrows indicate the cell wall of the cell referred as a sporangium (A, A*), long thick arrows point at gemmae (B, C) and thin arrows indicate the short, thick ring-like cell connections (G–I). Scale bars = 10 µm.

(Figs 2A–B; 7B–D; 9B; 10A). A characteristic feature of the clusters is that they are formed by the smallest groups of four cells and remain connected to a common base or their common threat-like, long stalk (Figs 6E–L; 7A). Most clusters contained only older cells with more homogenous content and uniform colour (Figs 2A, B, D–F; 7A–D; 8D–G; 9H; 10A), but clusters of both types of cells with net-like and homogenous content were often seen together (Fig. 7E).

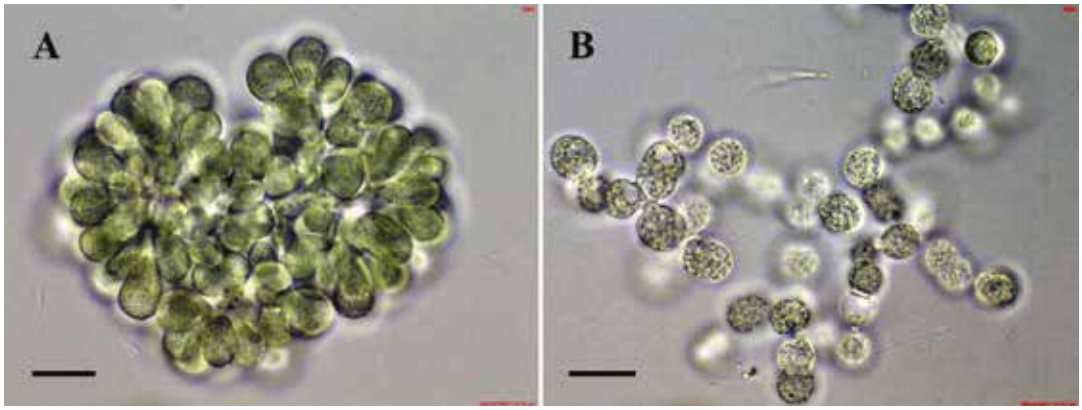


**Figure 10.** *Cyanocystopsis kitagatae* gen. et sp. nov. under high (100×) LM magnification and immersion. **Type.** Complex cluster of claviform cells with homogenous content (A); Branched filamentous stage with spherical cells and elongated dividing cells with net-like content and visible connections between the cells (B). Scale bars = 10 µm.

According to our observations, it seems that the basis of the initial 4-celled clusters (Fig. 6E–H) was formed by the ring-like or cap-like cell wall remnants of the dividing filamentous cells (described above). These remnants elongate to form long, colourless, thread-like structures with their age and development (Figs 6K–L; 7A). First, they keep the clusters together, but later they dissolve (Figs 6E–L; 7A), ensuring the disintegrating of the complex clusters to smaller ones.

Although rarely, we observed new transversal divisions at the top of claviform cells in well-formed dense clusters (Fig. 9B, C). After the newly formed spherical cells (5–6–6.5 µm in diameter) are detached, they divide transversally and form a filamentous stage (Fig. 9D–I). Interestingly, thick cell wall remnants were well visible during the division to the filamentous stage (Fig. 9G–I). The filaments formed in this way contain cells of homogenous content (Fig. 9I). Such transversal divisions and filamentous stages were rarely seen, suggesting that this vegetative reproduction through structures resembling unicellular gemmae is not the single way of reproduction of the studied species.

After thorough investigation of 21 microscopic slides, we managed to see free-floating small single and dividing cells of different dimensions and shapes on four more different slides, which were taken by us as spores of this species. The smallest visible spore was 3 µm and further cells increasing in diameter to 4, 5 and 6 µm were seen (Fig. 8A–F). These spores restore the filamentous stage through binary fissions (Fig. 8) and we can only suppose that they were released by the claviform cells.

In a single specimen of a well-developed cluster, we saw a group of four smaller cells within a common cell wall of a cell with type and dimension similar to other cells in the cluster (Fig. 9A). Due to the lack of more findings of such cells, it could be only tentatively supposed that it represents a sporangium. Two peculiar structures with a larger upper cell and a supporting lower cell were found in another cluster (Fig. 8H). We mention them here, but at this stage of development could not certainly refer them as sporangia.

In some observations, clusters of claviform cells and filaments at different stages were seen together (Figs 2C–F; 8G, H; 9I), but generally the clusters of different density prevailed (Fig. 2A–B). Similar claviform cells were described as sporangia mainly for the genera *Cyanocystis*, *Dermocarpa*, *Dermocarpella* (Dermocarpellaceae) and *Chamaecalyx* (Hyellaceae), which differ in the way of

opening and spore release (Komárek & Anagnostidis 2008). However, these genera were interpreted as epiphytic, without a specific vegetative filamentous stage, with closely situated sporangia, when each sporangium is attached separately to the substratum, whereas the alga described here was found in the phytoplankton in two different stages: filamentous and complex bundles of tetrads of claviform cells. At first apperance, it resembles the genus *Cyanocystis*. Therefore, we refer it to a new genus *Cyanocystopsis* with species epithet *kitagatae* originating from the type locality, the tropical crater lake Kitagata (Uganda, Africa).

At present, we have to note that the alga was found in a small, hypersaline and hypertrophic tropical crater lake. Therefore, it is possible to suppose the endemic character of this species, but only future studies with more observations can reveal its real distribution.

## Taxonomic description: Latin and English diagnoses

Genus ***Cyanocystopsis*** M.P. Stoyneva-Gärtner, G. Gärtner, B. Uzunov & J.-P. Descy gen. nov.

Cellulae globosae in filamentis elongates vel brevioribus consociatae, contentu reticuloso, initio uniseriatae, posterius filamenta elongatis et ramose divisiones succedaneis; partes membranae residua; paulatim cellulae claviformae crescentes, contentu reticuloso, praetere plusminusve homogeneo. Cellulae claviformae consociatae divisiones longitudinales, familias pluricellulares conjunctae per stipites communis. Familias numerosis quaternis cellulae claviformae compositae. Multiplicatio filamenta sporas divisione transversalis, raro per cellulas vegetativas (cellulae singulae vulgo gemmae) de apice cellulae claviformae post divisionem transversalis.

Affinis *Cyanocystis* Borzi sed differt structuram filamentas et cellulas claviformes, coloniae libere natantes, planctonicae.

**Typus.** ***Cyanocystopsis kitagatae*** M.P. Stoyneva-Gärtner, G. Gärtner, B. Uzunov & J.-P. Descy spec. nov.

Cellulae globosae, 6–6,5 µm diametro in filamentis, contentu reticuloso, posterius elongates (10–12 µm) per divisionem in duas partes et ramification. Partes membranae residua. Cellulae claviformae (8–)10–(12) µm longae, divisio longitudinalis in familias consociatae, 45–85–100–125 µm diametro, sed compositae in quaternis per stipites. Multiplicatio filamenta sporas divisionem transversalis, raro per cellulas vegetativas (gemmae, 5–6–6,5 µm diametro) de apice cellulae claviformae.

Ceterum ut in genere.

**Iconotypus.** Fig. nost. 10A, B.
Area geogr.: lacus Kitagata, Uganda, Africa.
Hab.: In aquis stagnalibus, coloniae planctonicae.
Collectores: Cédric Morana, Marc-Vincent Commarieu, Alberto Borges; 2017-04-08.
Species conservanda in collectionem algarum, Universitatis Sofia, Bulgaria.

Genus ***Cyanocystopsis*** M.P. Stoyneva-Gärtner, G. Gärtner, B. Uzunov & J.-P. Descy gen. nov.

**Description.** Filaments with spherical cells with net-like (?keritomic) content, short or long, firstly uniseriate, elongating through binary divisions. Later filaments ramificate with consequently increasing density through specific successive division and visible cell-wall remnants, with branches lying in more than one plane and a subsequent formation of claviform blue-green

cells. Claviform cells firstly are with net-like content, but soon become homogenous. They divide mainly longitudinally, in multiple consecutive divisions, forming complex clusters, but remain connected on a common stalk. Each cluster contains numerous typical small groups of four. Single spherical spores restore the filamentous stage by binary transversal divisions. More rarely, it can develop through multiple divisions of a spherical cell released from the top of the claviform cell after its single transversal division. Free-floating in the phytoplankton of a small tropical lake.

The present genus differs from *Cyanocystis* Borzi mainly by the presence of two different stages in the life cycle, by its peculiar division and reproduction as well as by free-floating mode of life in the limnoplankton.

**Type species.** *Cyanocystopsis kitagatae* M.P. Stoyneva-Gärtner, G. Gärtner, B. Uzunov & J.-P. Descy sp. nov.

Type locality: Lake Kitagata, Uganda, Africa

Habitat: Plankton.

Etymology: Combination of the Latin name of the genus *Cyanocystis* and suffix *-opsis*, which means 'looking like'.

***Cyanocystopsis kitagatae*** M.P. Stoyneva-Gärtner, G. Gärtner, B. Uzunov & J.-P. Descy sp. nov.

**Description.** Filaments with spherical cells with net-like (?keritomic) content, up to 6–6.5 µm in diameter. Filaments are short or long, firstly uniseriate, elongating through binary divisions, with cells reaching 10–12 µm length during division. Later filaments ramificate intensively through specific successive division and visible cell-wall remnants between the cells, with branches lying in more than one plane and a subsequent formation of claviform blue-green cells, (8)–10–(12) µm long. Claviform cells firstly are with net-like content, similar to the content of the filamentous stage, but soon become homogenous. They divide mainly longitudinally, in multiple consecutive divisions, thus forming complex clusters (45–85–100–125 µm in diameter), but remain connected on a common stalk in typical smallest groups of four. Single spherical spores (which almost immediately achieve the net-like content) restore the filamentous stage by binary transversal divisions. More rarely, the filamentous stage can develop directly through multiple divisions of a spherical cell (5–6–6.5 µm in diameter) with homogenous content, released from the top of a claviform cell after its single transversal division. Free-floating in the phytoplankton of a small tropical lake.

**Iconotype.** Figure 10A, B.
Type locality: Lake Kitagata, Uganda, Africa.
Habitat: Plankton.
Collected by: Cédric Morana, Marc-Vincent Commarieu, Alberto Borges; 8th April 2017.
Etymology: The species epithet *kitagatae* refers to the first locality, where the species was found – tropical crater lake Kitagata (Uganda, Africa).
Type species is deposited in the algal sample collection of Sofia University 'St Kliment Ohridski'.

## Acknowledgements

Water samples were collected by Cédric Morana, Marc-Vincent Commarieu and Alberto Borges (University of Liège) in the frame of the HIPE project, funded by the Belgian Science Policy Office (BELSPO, Brussels, Belgium) under the BRAIN program (BR/154/A1/HIPE).

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