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Tropical chroococcalean morphospecies (Cyanobacteria) in Cuban waters

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Abstract. Three natural populations of chroococcalean cyanobacteria were found in water samples collected from Cuban localities. Based on their morphological characteristics, the populations were composed by 3 morphospecies, which can be identified with *Aphanothece variabilis* (Schiller) Komárek, *Chroococcus* cf. *major* Komárek et Komárková-Legnerová and *Radiocystis fernandoi* Komárek et Komárková-Legnerová. According to the literature consulted these morphospecies are known from tropical and subtropical regions only. In the present paper descriptions, illustrations and comments about taxonomy, ecology and geographical distribution of each taxon are offered. *A. variabilis* was earlier found from different Cuban localities, but not from Central part of Cuba. *Ch. major* and *R. fernandoi* are new-records to the Cuban flora.

Key words: Cyanobacteria, natural populations, taxonomy, ecology, distribution

Resumen. **Morfoespecies chroococcales tropicales (Cyanobacteria) en aguas cubanas**. Tres poblaciones naturales de cianobacterias cocales fueron encontradas en muestras colectadas en varias localidades cubanas. De acuerdo con sus caracteres morfológicos, las poblaciones, estaban compuestas por 3 morfoespecies que pueden ser identificadas como *Aphanothece variabilis* (Schiller) Komárek, *Chroococcus* cf. *major* Komárek et Komárková-Legnerová y *Radiocystis fernandoi* Komárek et Komárková-Legnerová. Según la literatura consultada, estas morfoespecies se conocen para las regiones tropicales y subtropicales. El presente artículo ofrece descripciones, ilustraciones y comentarios acerca de la taxonomía, ecología y distribución geográfica de cada taxon. *A. variabilis* se conocía anteriormente para otras localidades de Cuba, no para la parte central de Cuba. *Ch. major* y *R. fernandoi* son nuevos registros para la flora cubana.

Palabras-clave: Cianobacterias, poblaciones naturales, taxonomía, ecología, distribución

Introduction

The conflict between traditional taxonomy schemes based on morphology and new molecular genetic information has led to a situation of great complexity. Phylogenetic trees obtained after gene sequence analysis have provided, of course new insights into the taxonomy and systematic of the cyanobacteria, very often with conclusions disagreeing with the classical taxonomy. For this reason, some authors mean, that the "species" category in cyanobacteria could not be justifiable (Castenholz 2001). However, many recognizable morphotypes, which were usually classified as species in traditional sense, really exist and

repeatedly were found in different regions (Komárek & Komárková, 2002). They can be characterized at present, only eventually as morphospecies, or at least, as morphotypes, that probably belong to one genotype having similar ecology. Such organisms with distinct phenotype and ecophysiological features cannot be completely omitted, their identification, in fact, is useful and necessary for hydrobiological sciences.

The taxonomy and systematic of these cyanobacteria is also problematic: the number of morphological features is scarce, and by other hand, the occurrence of several morphotypes have originated different meanings.

The importance of the ecology and geographical distribution of the chroococcalean cyanobacteria was shown by Komárek (1995) and Komárek & Komárková-Legnerová (2007), taking into account, that several morphotypes occur in characteristic biotopes in delimited geographical areas, e.g. in tropical and subtropical zones. Some of these morphs were supported by molecular analysis (Komárková et al. 2010).

After Hoffmann et al. (2005) polyphasic studies of cyanobacteria are broad extended. These studies are based on phenotypic characterization of natural and culture populations, electron microscopy research and gene sequence analysis, that means a comparison of ultrastructure of genetically identified items with their morphological expression.

In spite of the phenotypic characteristics are no conclusive for establishment taxonomical entities, the evaluation of morphospecies is important from an ecological point of view, but generally can be made only on the basis of natural populations. The preliminary phenotypic and ecological characterizations of natural populations are necessary for both further ecological and systematic research.

The present paper contributes to the recognition of three unicellular cyanobacteria and offers information about morphological variability, taxonomy, ecology and geographical preferences of the involved taxa. The results are based on optical microscopy studies of natural populations from some Cuban localities following the classification system proposed by Komárek et al. (2014).

Materials and Methods

The samples were collected from 3 localities: 1) Plankton samples from Laguna Ancón, a shallow coastal lagoon (1-1,5 m deep, salinity 42,2-42,8 ups), near “Hotel Ancón”, Casilda, Sancti Spiritus (center of lagoon at 21º 43' 55" N, 79º 59' 39" W) , taken in June 2015; 2) detritus at bottom from a shallow occasional pool (10 cm deep) in Cienfuegos Meeting Square, collected in March and April 2013; 3) Plankton samples from Hanabanilla Reservoir, Villa Clara (center of lagoon at 22º 3' 63" N, 80º 3' 12" W), collected in March and November 2014. The pool samples were taken from detritus at the bottom and later transferred to 50 mL plastic flasks. The plankton samples were collected with a phytoplankton net with 20 µm pore diameter stored in 250 mL plastic flasks. All samples were fixed with formaldehyde at 3% (total solution) and studied in a Laborlux, Leica-Leitz microscope. The microphotographs were taken with an Axiocam Carl Zeiss digital camera. The specimens were also documented by drawings. We are following in this paper the classification system proposed by Komárek et al. (2014).

Results

Ancón Lagoon: In this lagoon a water bloom formed by floating blue green agglomerations (mats) covering almost the entire water body was observed. It was composed mainly by a colonial cyanobacteria. The colonies were spherical to irregularly elongated, mucilaginous with margins sharply delimited. The cells were rod-shaped to almost cylindrical, usually agglomerated in the center of the colonies. According to these features the specimens can be included into to the genus *Aphanothece* subgen. *Cyanogastrum* (Schiller) Komárek 1995, very close to *A. variabilis* (Schiller) Komárek 1995.

Description: Aphanothece variabilis (Schiller) Komárek 1995 (= *Cyanogastrum variabile* Schiller 1956) (Figs. 1-3).

Colonies (Fig. 1) are spheroidal, irregularly elongated up to kidney-shaped, mostly solitary or formed by 2-3 subcolonies, surrounded by their own envelopes into the general matrix, microscopic or in macroscopic floating aggregates, 44, 4-88,8 x 44,4-66,6 µm, with cells in 1-2 layers which are densely agglomerated in the center or sometimes in the colonial margin. Colonial mucilage is widened, exceeding cell agglomerations, homogeneous or mostly double-layered, colorless, with a sharply delimited margin. Cells are broad oval, elongated oval, but mostly rod-shaped up to almost cylindrical, (4,4) 8,6-14,0 (22,2) x (4,4) 5,6 (13,3) µm, with rounded ends and with pale blue green, homogeneous, fine granulated cell content. Reproduction by binary fission in one plane. Propagation: by fragmentation of colonies.

Shallow pool in Cienfuegos: In detritus, at bottom of the pool, a very reach population of a *Chroococcus* morphoespecies was found. According to its features: type of colonies, structure of colonial mucilage, cell shapes and dimensions, our population could be included into *Ch. major* Komárek et Komárková-Legnerová 2007.

Description:–*Ch*. cf. *major* Komárek et Komárková-Legnerová 2007 (Fig.4).

Colonies solitary, spherical to ellipsoidal, with 2-4 cells (sometimes solitary cells also occurs), with distinct delimited, refractive, colorless, mostly homogeneous or sometimes slightly lamellated colonial envelopes. Cells hemispherical or irregularly spherical, rounded, 14-22,4 µm in diameter, dark blue green, usually with 2-4 granules dispersed inside the cells. Reproduction: by cell division in at least 3 planes, perpendicular to one another. The cells divided by binary fission do not reach the original shape before next division. Propagation: by liberation of cells or group of cells.

Hanabanilla Reservoir: In plankton samples from Hanabanilla Reservoir, a colonial cyanobacteria was frequently found. The most distinguishing feature of these specimens was the arrangement of cells in irregular short rows into the colonies, which are evidently oriented to the center of the colonies. According to this, it corresponds with the genus *Radiocystis* and identical to *R. fernandoi* Komárek et Komárková-Legnerová 1993.

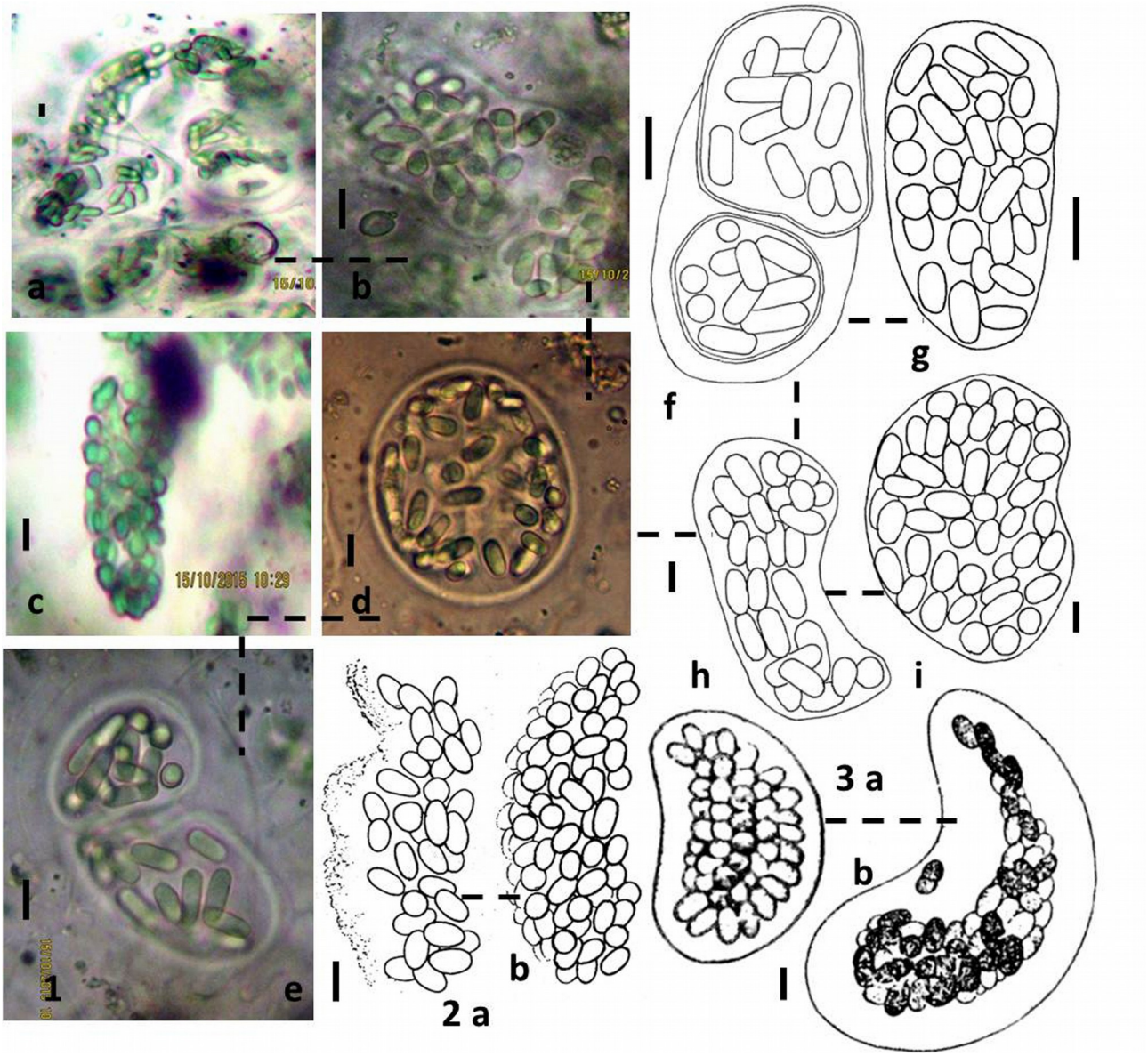


Figure 1. *Aphanothece variabilis*, a-i) Population collected from Ancón Lagoon, variability of cells and colonies; j-k) (After Komárek 1995); l-m (after Schiller 1956). Bars = 10 µm.

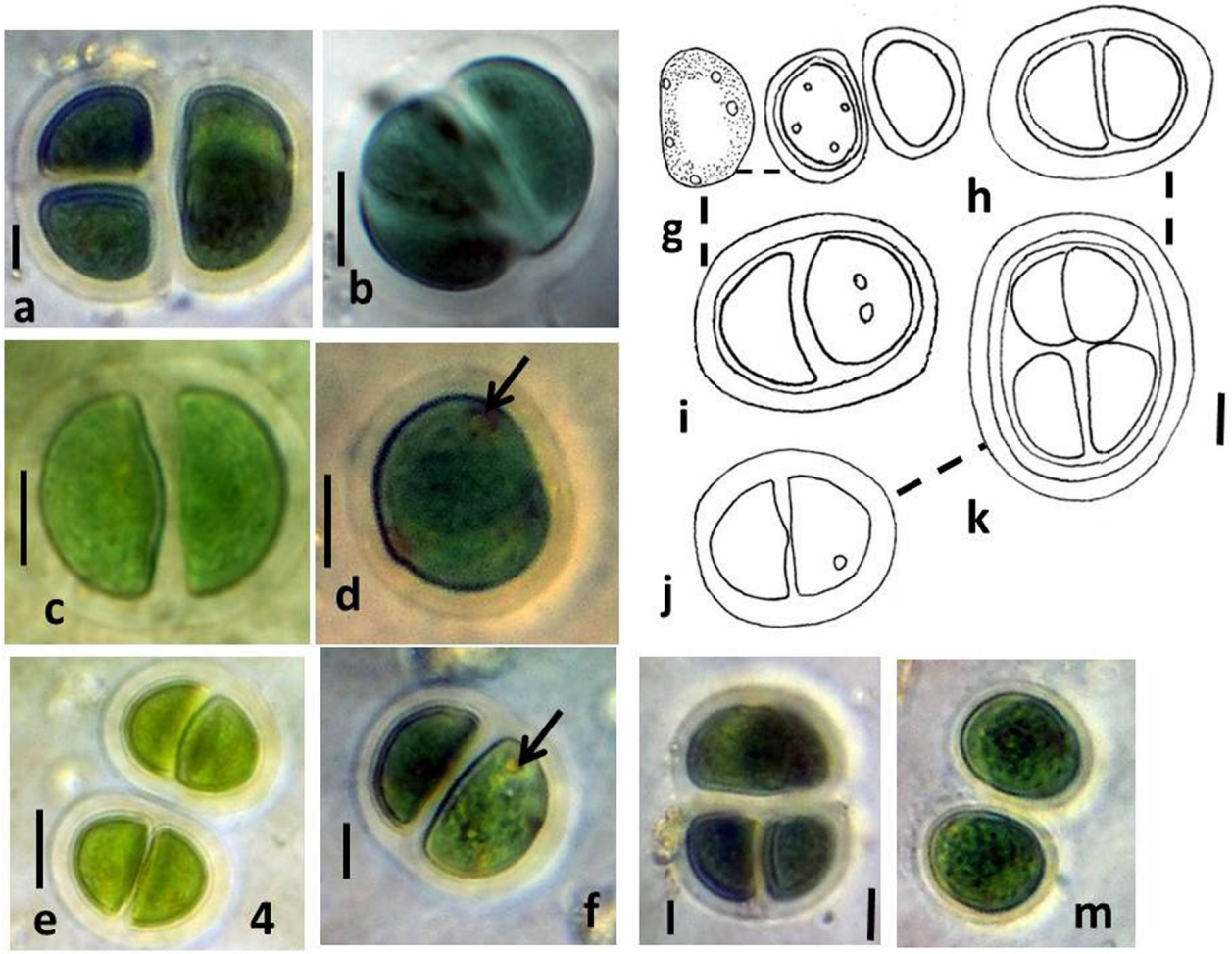


Figure.4. *Chroococcus* cf. *major*, Cuban population collected from a shallow pool at Cienfuegos Meeting Square, a, e, k, l) four-celled colonies; c, f, h, i, j, m) two-celled colonies; d, g) solitary cells; b) cell division. Arrows = granules. Bars = 10 µm

Description: R. fernandoi Komárek et Komárková-Legnerová 1993 (Fig.5).

Colonies spherical or irregularly spherical, surrounded by a colorless, diffluent or distinct mucilaginous envelop. Cells spherical, 6-8,5 µm in diameter, mostly arranged in short, (sometimes two cells only) rows, which are oriented to the center of colony. Cell content pale or dark blue green, usually with aerotopes. Reproduction: by transversely cell division in one plane in successive generations, later perpendicularly to the radiating rows. Propagation: by disintegration of colonies.

Discussion

Cyanogastrum variabile was described by Schiller (1956) from costal water bodies in Isla Las Aves (Northern Venezuela). The author considered the agglomeration of cells in the center of the colonies as good generic feature, therefore the genus *Cyanogastrum* was created, however it was not accepted by Komárek (1995), but included into *Aphanothece*. According to Komárek et al. (2011, 2014), the genus appears to be monophyletic, but sequence data for the type species must be confirmed. Based on the cell disposition inside the colony, observed also in other tropical *Aphanothece* species (*A. hegewaldii* Kovačik 1988 and *A. comasii* Komárková-Legnerová et Tavera), this later author proposed it as a new status at subgenus level: *Cyanogastrum* (Schiller) Komárek 1995. *A. comasii* was observed first in Cuban samples, but it was described from Lake Catemaco, Mexico (Komárková-Legnerová & Tavera 1995). *A. variabilis* is known from several Cuban localities with fresh, brackish or salty waters (Komárek 1995, Comas 2009, Dobal et al. 2012), occurring in the metaphyton among other algae and cyanobacteria, in detritus or forming floating agglomerations in shallow water bodies. According to Komárek & Komárková-Legnerová (2007), *A. variabilis* is a

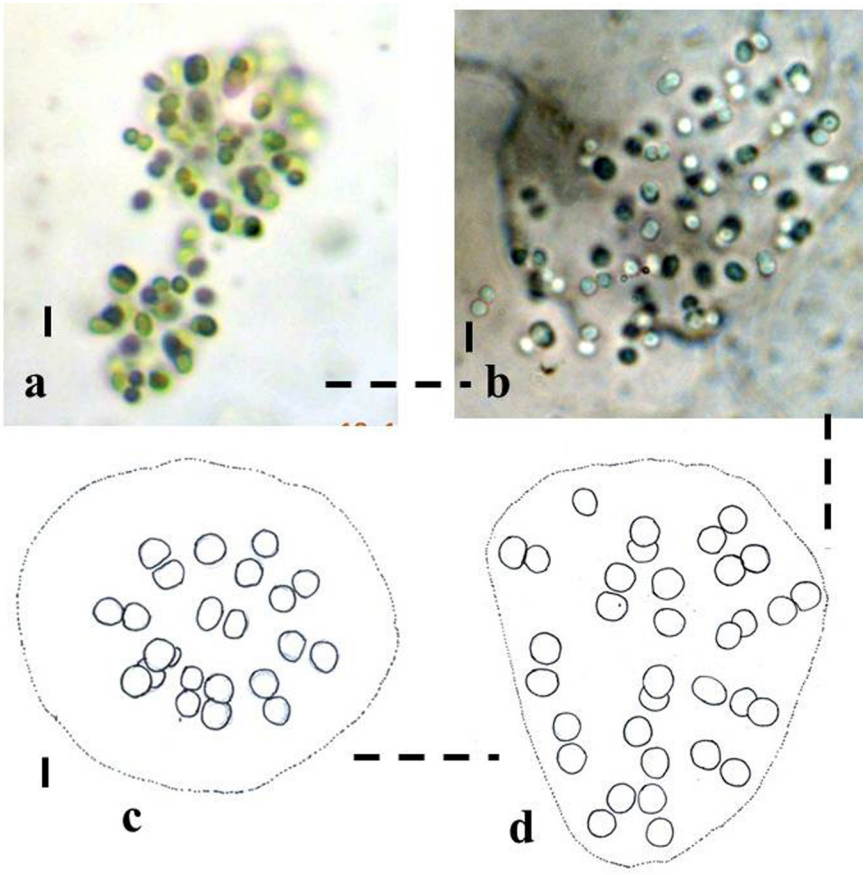


Figure. 5 a-d) *Radiocystis fernandoi* from Hanabanilla Reservoir, Cuba. Bars= 10 µm

tropical morphospecies, not frequently found. It is, up to date, known from Belize, Cuba and Venezuela. Mareš (2006), registered the species from the Everglades (Florida, U.S.), but his specimens belong probably to a different morphotype.

Schiller (1956) and Komárek (1995) observed solitary cells together with the characteristic elongated to kidney-shape colonies (see Komárek & Anagnostidis, 1999, pag. 89, Fig. 82). The cell dimensions of the Cuban specimens (Komárek 1995) were 8-10 x 4,8-5,4 µm. Komárek & Komárková-Legnerová (2007) found natural populations of this species in Belize marshes with low to high conductivity, however they found spherical colonies only and the cells were a little bigger (8,6-12,4 x 5,6 µm). Similar colonies are formed in *Cyanoaggregatum brasiliense* Werner et al., but the cells are smaller in this genus (2,4-3,8 x 1,4-2,0 µm) and disposed in single flat layer in irregular rows forming a mosaic-like pattern, slightly below the surface of the mucilaginous colonial envelope (Werner et al. 2008).

Our population could be identified with *A. variabilis*, but presents some particularities: a) the colonies can be formed by subcolonies, b) the cells are evidently bigger: (4,4) 8,6-14,0 (22,2) x (4,4) 5,6 (13,3) µm, mostly almost cylindrical and c) it was found from a lagoon with high salinity forming a typical water bloom. Probably its occurrence in plankton is secondary, growths at bottom and later emerges in floating agglomerations. Nevertheless, we prefer consequently identify this population with *A. variabilis.* This species up to date is not supported by molecular analysis.

The genus *Chroococcus* is distributed over the world, few of its species are supported by molecular analysis (Komárková et al. 2010), the majority of them still remain as morphospecies, which grow in different biotopes. Its taxonomy is confused, particularly of those metaphytic natural populations growing in tropical or subtropical water bodies. Such is the case of populations identified as *C. turgidus* (Kützing) Nägeli 1849, a species which was described from cold moors and acidic swamps in Central Europa, occurs also metaphytic in non-polluted waters, in littorals (very rare secondary in plankton) of clear water bodies in temperate zones (Komárek & Novelo 1994; Komárek &

Anagnostidis 1999, Komárek & Komárková-Legnerová, 2007). Nevertheless there are very similar morphotypes, growing in tropical countries but under very different ecological conditions. They are morphologically similar to *C. turgidus*, but appear in warm, slightly alkaline tropical waters (swamps, springs and creeks). These populations, according to Komárek & Novelo (1994), are identical with *Ch. pulcherrimus* Welsh 1965. In fact, Cuban similar populations were named as *Ch. turgidus*, but without doubt, they belongs to *Ch. pulcherrimus* (Comas 2009).

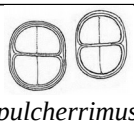
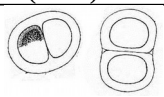
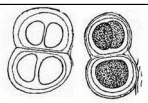
Seven morphospecies related to *Ch. pulcherrimus* were described from alkaline marshes in Belize (Komárek & Komárková-Legnerová 2007), which in our meaning, are very difficult to distinguish one to another. Our population is compared with those morphospecies more related (Table I), in some characteristics resemble *Ch. major*, *Ch. occidentalis* Komárek et Komárková-Legnerová 2007 or *Ch. pulcherrimus.* The occurrence of solitary cells is frequent in our samples but the colonies are mostly 2-4 celled. According to these features our species looks like *Ch. occidentalis*. In *Ch. pulcherrimus* and *Ch. major* the solitary cells are very rare. The homogeneous to very slightly lamellated colonial envelopes correspond better to *Ch. occidentalis* or to *Ch. major*. *Ch. pulcherrimus* shows conspicuous lamellated mucilage. The cells in our population are smaller than those of *Ch. occidentalis*, or *Ch. pulcherrimus*, but they are closer to *Ch. major* (Table I). Several granules centrally located inside cells are typical to *Ch. major*. The cells in Cuban specimens present 2-4 sparse granules, not centrally located. Then. the presence and disposition of granules inside the cells are not be good diagnostic features. Because the Cuban population appear in quite different ecology (eutrophied waters), we prefer name as *Ch*. cf. *major*. Typical *Ch. major* was only known from the alkaline marshes of Belize, in unpolluted waters with low to high conductivity. This morphospecies was not found in Cuba before. For its definitive taxonomical identity further studies are required.

According to Komárek et al. (2014) only limited unclear and raw molecular data are available for *Radiocystis*. *R. fernandoi* was described from Brazil (Komárek & Komárková-Legnerová, 1993) and up to date, known also from Indonesia, Sri Lanka and South Africa (Komárek & Anagnostidis, 1999) and Uruguay (Ferrari et al. 2011). Our Cuban population is quite identical with the original description and also correspond with the findings published by Sant´Anna et al. (2004) from Brazil. This morphospecies is a new report to Cuban flora.

Conclusions

Chroococcus cf. *major* and *Radiocystis fernandoi* are new findings to the Cuban flora, *Aphanothece variabilis* was known from others localities in the country but not from Central part of Cuba.

Table I. Comparison of *Chroococcus* cf. *major* with other related morphospecies based on main characteristics according to Komárek & Komárková-Legnerová (2007).

	 *pulcherrimus*	 *occidentalis*	 *major*	 cf. *major*
Colonies	2-4-celled	Mostly unicelular or 2-4-celled	Solitary or 2-4-celled	Solitary or 2-4-celled
Colonial envelope	Always lamellated	Homogeneous or rarely slightly lamellated	Finely lamellated	Homogeneous or rarely slightly lamellated
Cell diameter µm	12,4-28,6(42,0)	(9,8)14,5-52,0(64,5)	(7,4)11,2-21,0	14-22
Granules	-	-	In the center	dispersed
Ecology	Metaphyton or detritus of eutrophic waters with medium to higher salinity	Metaphyton of slightly eutrophied marshes	Metaphyton of unpolluted shallow pools, rarely in slightly saline marshes	In detritus of hardly eutrophied shallow pool
Distribution	Pantropical	Porto Rico and Belize	Porto Rico, Belize and Florida	Cuba

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