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FIRST RECORD OF *CYANOPHORA PARADOXA* KORŠIKOV (*Glaucocystophyta*) IN ITALY

SUMMARY

The occurrence of *Cyanophora paradoxa* Koršikov (Glaucocystophyta) in a small temporary pond in Sicily is documented. The monadoid *Cyanophora paradoxa* is seldom found in freshwater water bodies of the world and represents a major model organism in the evolution studies. The finding represents the first record of this remarkable planktic species in Italy. The limnological characterization of the pond, as well as the composition of the phytoplankton assemblage at the time of finding, are illustrated.

RIASSUNTO

Primo rinvenimento in Italia di Cyanophora paradoxa *Koršikov*. La rilevante importanza evolutiva della glaucocistofita monadoide *Cyanophora paradoxa* Koršikov, considerata come “fossile” chiave a supporto della teoria endosimbiotica, non corrisponde di fatto alle scarse conoscenze sulla sua distribuzione e sulle sue peculiari esigenze ecologiche. Rinvenuta esclusivamente nel plancton di acqua dolce, è stata raramente segnalata. Nell’ambito di uno studio limnologico, condotto su oltre 100 corpi idrici siciliani temporanei, è stata riscontrata la sua presenza in un piccolo stagno eutrofico siciliano (Gorgo di Rebuttone), alimentato esclusivamente da acque piovane e saltuariamente adibito ad abbeveratoio per il bestiame. Questa nota, che segnala il primo ritrovamento in Italia di *Cyanophora paradoxa*, contribuisce all’ampliamento delle informazioni sull’ecologia di questa peculiare alga planctonica. Lo studio è comprensivo della caratterizzazione limnologica del corpo idrico e della struttura tassonomica dell’associazione fitoplanctonica al momento del rinvenimento.

INTRODUCTION

The monadoid microalga *Cyanophora paradoxa* Koršikov belongs to the Phylum Glaucocystophyta Kies & Kremer (Glaucophyta Skuja). They are often considered "enigmatic", as being a polyphyletic taxon of photoautotrophic protists, which are characterized by the presence of peculiar plastids, called cyanoplasts, which were formerly considered as endosymbiotic cyanobacteria called cyanelles (KIES, 1992).

Cyanoplasts show structural, biochemical and physiological features intermediate between those of cyanobacteria and other photosynthetic organisms (HALL & CLAUS, 1963; SCHENK, 1994). In particular, they retain a peptidoglycan layer between the delimiting membranes similar to that of cyanobacteria but lacking around the plastids of other photoautotrophs. Moreover, cyanoplasts' genome is very similar to that of other plastids, whereas, the pigments and the delimiting membranes are typically cyanobacterial (BHATTACHARYA *et al.*, 1995; DOUGLAS & RAVEN, 2003; HELMCHEN *et al.*, 1995; SATO *et al.*, 2005).

Thus, the organisms grouped in the Glaucocystophyta offer a living support to the endosymbiotic theory, to the origins of chloroplasts and to primary endosymbiosis. Particularly, *Cyanophora paradoxa*, considered the "Celacanthus" of endosymbiosis (MCFADDEN, 2001), is the main key organism supporting the theory of endosymbiosis. Nevertheless, its evolutionary importance does not match the scarce knowledge on its distribution and ecological requirements.

As other glaucocystophytes, *C. paradoxa* has been rarely collected in the field and is exclusive for freshwaters (REVIERS, 2003). In Europe, to our knowledge, it was recorded in UK (WHITTON, 2002), Switzerland (BOURRELLY, 1985) and Germany (KIES, 1992).

Thus, in the framework of a limnological investigation carried out on a Mediterranean temporary pond, the finding of *C. paradoxa* in Sicily represents, as shown by BARONE & NASELLI FLORES (2004), its first record in Italy. Moreover, to our knowledge, it is the first glaucocystophyte recorded in Italy, though *Glaucocystis nostochinearum* has been recorded in North Tyrol (GÄRTNER & INGOLIC, 2001).

THE RECORD

Cyanophora paradoxa was recorded in the "Gorgo di Rebuttone", a natural Mediterranean temporary pond fed by rainwater with a flooded period generally spanning from October to July. It is located near Palermo (38° 01' 42" N; 13° 19' 36" E) at 700 m a.s.l. by the side of the road to Piana degli

Albanesi and lays on calcareous rocks covered by a clayey layer. At maximum capacity in winter season (Fig. 1A), it has an oval shape with axes 54 m and 28 m. Maximum depth reaches 1 m in the middle of the water body. The pond is used as a drinking water supply for cattle and horses and in the summer dry period (Fig. 1B) hosts a therophytic community where these animals feed on.

Fig. 1 — Gorgo di Rebuttone. A = Winter; B = Summer.

Large amount of organic matter are supplied by the flooded terrestrial vegetation as well as by the dejection of cattle.

A limnological investigation was carried out in the pond during the flooded period during 2003 and 2004 (for the methods used see MARRONE *et al.*, 2006). Phytoplankton samples were collected sub-surface and subsamples were stored both alive for identification aims, and fixed with Lugol's iodine solution for counting.

In the study site, temperature values ranged between 8.5 (± 0.3) °C in December and 33 (± 1.2) °C in July. Conductivity records (at 20 °C) varied between 143 (± 11) µS cm^{-1} in January and 400 (± 32) µS cm^{-1} in July/October (at the end and at the beginning of the water phase). Strictly linked to photosynthetic activity, pH values ranged between 7.1 (± 0.1) and 8.7 (± 0.3). Starting from the end of November to March-April, *Glyceria fluitans* (L.) R. Brown is the most abundant aquatic plant. Among metaphytic algae, *Spirogyra* sp. forms large bundles all around the pond. The pond represents an important reproductive site for amphibians [*Discoglossus pictus* Otth and *Bufo bufo* (L.)]. In spite of its temporality, a large amount of fish (*Gambusia holbrooki* [Girard] and goldfish) were found in the pond. These were likely stocked by people living in the surroundings. At the end of the flooded period they die and give a further contribution to the organic matter in the pond.

Cyanophora paradoxa was collected on 4 August 2004. Its finding occurred immediately before the complete drying of the pond, which in 2004 was delayed because of the intense spring precipitation. In that period, only a few water was still present in a hole: a 60 cm in diameter and 50 cm deep dig by shepherds to maintain a water source for cattle. Water temperature was 31 °C and conductivity (at 20 °C) reached 440 µS cm^{-1}.

Cells of *C. paradoxa* (Fig. 2) were found during an intense bloom of Chlorococcales (*sensu* Komarek & Fott) and contributed 2% to the total density ($120 \cdot 10^6$ cell l^{-1}). Their lengths ranged between 8 and 10 µm, widths between 4 and 5 µm and they had a thickness of 3-4 µm. The cyanoplasts were 2-3 µm in diameter. Observations carried out on living samples showed the organisms actively swimming and they were easily distinguishable from the other eucaryotic microalgae due to the bright blue-green colour of their cyanoplasts.

The phytoplankton assemblage (Tab.1) showed a high species richness and was co-dominated by *Actinastrum hantzschii* Lagerheim, *Ankistrodesmus* sp. and *Diacanthos belenophorus* Koršikov. The latter is a rarely recorded chlorophyte as well as the co-occurring *Keratococcus bicaudatus* (A. Braun) Boye-Petersen. Other interesting and rarely recorded taxon co-occurring with *C. paradoxa* and belonging to the Division Xanthophyta was *Centritractus belenophorus* Lemmermann.

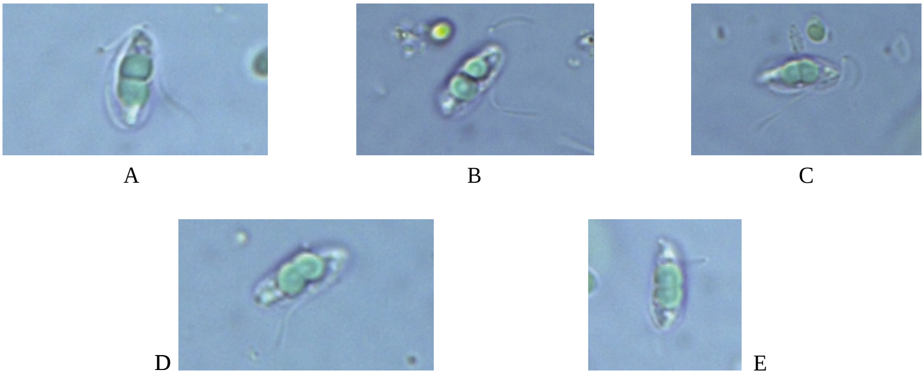

Fig. 2 — *Cyanophora paradoxa* (630x). A = ventral view; B = dorsal view; C = lateral view; D, E = prominent cyanoplasts.

TAXONOMY, MORPHOLOGY AND ECOLOGY OF *CYANOPHORA PARADOXA*

The most recent taxonomic trees of Eucaryotes place the phylum Glaucocystophyta into the super-group "Plantae", along with the red algae, the green algae and the terrestrial plants (Keeling *et al.*, 2005). A recent classification scheme of the eucaryotes, proposed by the International Society of Protistologists (Adl *et al.*, 2005), put the glaucocystophytes into the group Archaeplastidia. This group is characterised by the presence of a photosynthetic plastid with chlorophyll *a* deriving from an ancestral event of primary endosymbiosis with a cyanobacterial cell. The most reliable molecular trees tend to favour the hypothesis of glaucophytes divergence before those of red- and green- algae (Keeling, 2004).

The phylum Glaucocystophyta (Kies & Kremer, 1986) has only one Class (Glaucocystophyceae), three Orders (Cyanophorales, Gloeochaetales, Glaucocystales), three Families (Cyanophoraceae, Gloeochaetaceae, Glaucocystaceae), three Genera (*Glaucocystis*, *Cyanophora*, *Gloeochaete*) and, up to now, 14 species. In particular, the genus *Cyanophora* includes three species: *C. paradoxa* Koršikov, *C. tetracyanea* Koršikov and *C. biloba* Kugrens, Clay, Meyer & Lee.

C. paradoxa cells are commonly obovate (Fig. 3), with a dorsoventral simmetry. According to Whitton (2002) cell length range between 8 and 12 µm, their width is 5-8 µm, and their height is 2-3 µm. The dorsal side is rounded, whereas the ventral one is flat. Two heterocont, heterodynamic flagella originate from a ventral, subapical depression (furrow). One flagellum protrudes forward from the cell apex, while the other emerges from this same location but instead points towards the posterior portion of the cell and partially lies in the

Table 1
Taxonomic composition of the phytoplankton assemblage.
** = density value between 5 and 10%; ** = density value between 10 and 30%.*
No asterisk indicate a relative density between 1 and 5%.

Glaucophytes
Cyanophora paradoxa Koršikov
Chlorophytes
Actinastrum hantzschii Lagerheim **
Ankistrodesmus spp. **
Chlorogonium elegans Playfair
Closterium sp.
Cosmarium spp.
Diacanthos belenophorus Koršikov **
Dictyosphaerium cf. *pulchellum* Wood **
Elakatothrix cf. *gelatinosa* Wille
Golenkinia radiata (Chodat) Wille
Gonium sp.
Keratococcus bicaudatus (A. Braun) Boye-Petersen
Lagerheimia sp.
Micractinium pusillum Fresenius
Monoraphidium spp.**
Oocystis spp.*
Pandorina sp.
Pediastrum spp.*
Quadrigula sp
Scenedesmus spp.**
Tetraedron sp.
Tetraedron caudatum (Corda) Hansgirg **
Treubaria triappendiculata C. Bernard **
Xantophytes (Mischococcales)
Centritractus belenophorus Lemmermann
Euglenophytes
Trachelomonas volvocina Ehrenberg
Trachelomonas hispida var. *coronata* Lemmermann
Phacus triqueter (Ehrenberg) Dujardin
Lepocinclis ovum Ehrenberg
Euglena spp.
Cryptophytes
Cryptomonas sp.
Dinophytes
Gymnodinium sp.
Bacillariophytes
Navicula spp.

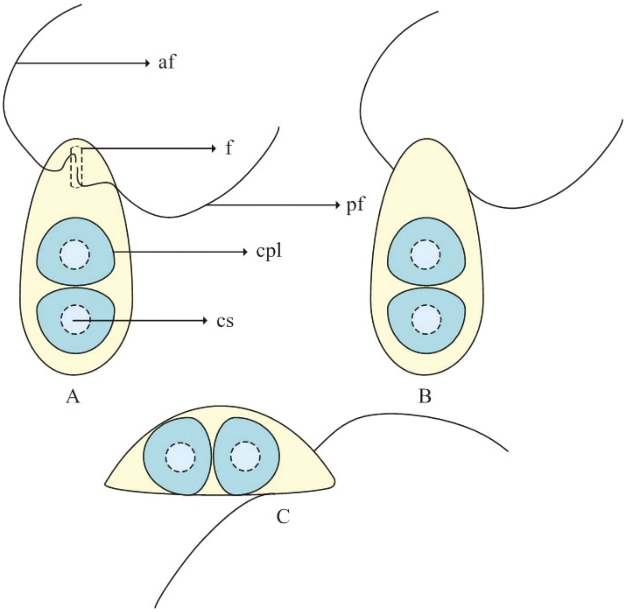

Fig. 3 — *Cyanophora paradoxa* drawings. A = ventral view; B = dorsal view; C = lateral view. Af = anterior flagellum; pf = posterior flagellum; f = furrow; cpl = cyanoplast; cs = carboxysome.

sulcus. Both show two tiny rows of fine fibrillar hairs, similar to those of green-algae. Two contractile vacuoles are located close to the flagella insertion. Although lacking a stigma or a phototactic orientation, the cells can swim away from bright lights using photoreceptors in the plastids (KUGRENS *et al.*, 1999).

Cyanophora paradoxa is obligatorily a photoautotroph organism. Penicillin inhibits the peptidoglycan synthesis and the cyanoplasts are digested. Nevertheless, the aposymbiotic organism can keep on dividing until storage reserves are present.

This organism has one, two or, rarely, 4-8 sub-spherical chloroplasts. The plastids are included in a typical vacuole and are delimited by two membranes, which in between retain a peptydoglycan wall. The peptydoglycan layer seems to have a role in maintaining a high osmolarity into the cyanoplasts (likely to favour their growth), as requested by the mechanism, which allows these organisms to concentrate inorganic carbon (RAVEN, 2003). Thylakoids are located peripherically; they are isolated and concentrically ordered as in the cyanobacteria.

The plastids contain chlorophyll *a* and two blue-coloured phycobiliproteins: the phycocyanin and the allophycocyanin. These, as well as in Cyanobacteria and Rodophyta, are assembled in phycobilisomes, which are small particles located on the external side of the thylakoids' membrane. Among carotenoid pigments, β-carotene, zeaxanthin, and β-cryptoxanthin are present but two typical cyanobacterial xanthophylls are absent: myxoxanthophyll and myxoxanthin.

The main storage polysaccharide is an extraplastidial branched polyglycan. Although very similar, it is less branched than that commonly found in cyanobacteria.

Each cyanoplast contains an electronically dense central body (carboxysome) and a central DNA ring, similar to the nucleoid of cyanobacteria. Carboxysomes contain RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) and serve as sites where carbon dioxide can be concentrated, thus improving the kinetics of carbon fixation.

The nucleus of *Cyanophora paradoxa* is located in the posterior part of the cell and has a horse saddle shape. Mitochondrial cristae are flattened as in the cryptophytes, rhodophytes and chlorophytes. They undergo open mitosis without centrioles and the mitotic fuse persists till the telophase.

C. paradoxa lacks a cell wall and it is bounded by a rather complex structure. The plasmatic membrane includes a system of vesicles, similar to the alveoles found in the dinoflagellates, subtended by a microtubular system. Protruding mucocysts are located beneath the plasmatic membrane.

Cyanophora reproduces by dividing longitudinally into two daughter cells. Cell division is asynchronic with cyanoplast division. Before dividing the flagellar apparatus is duplicated in a semi-conservative way. The cyanoplast division mode is intermediate between cyanobacteria and plastids (IINO & HASHIMOTO, 2003). Sexual reproduction has not been observed.

Among Glaucocystophyta, the monadoid genus *Cyanophora* is generally planktic in small and productive ponds and channels, whereas the coccoid *Glaucocystis* is metaphytic in channels, ponds and in the littoral zone of lakes. The palmelloid genus *Gloeochaete* is epiphytic on filamentous algae or on aquatic mosses and submerged macrophytes.

In particular, *Cyanophora paradoxa*, commonly found in shallow, eutrophic freshwater bodies, was first described from a sample collected in a small lake in Russia (KORŠIKOV, 1924). Among the other organisms belonging to the genus, *C. tetracyanea*, characterised by a bilobate shape and by the presence of four spherical cyanoplasts, was collected in a Russian river (KORŠIKOV, 1941) and in a Swedish lake (SKUJA, 1956) but its validity as a good species is controversial. *C. biloba*, showing a bilobate shape and two cyanoplasts in a permanently stopped cytokinetic stage, was found in a small and shallow temporary alpine pond in Colorado (KUGRENS *et al.*, 1999).

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