

S1. Complete morphological description of specimens studied

The living plants are light or yellow–green with distinctly light orange–brownish apices; this color had disappeared during herbarization (cf. Figs 1, S1, S2). The herbarized plants are green, green grey to light ash–colored in old parts. The degree of incrustation varies in specimens from different localities from calcite–unencrusted with slightly encrusted old parts (Ein Abu Natekina) to the moderately and heavily incrustated (Ein Shalala, Ein Yirka) with the lowest parts being the most encrusted.

Two forms of growth are presented in the collections. The first one is a medium–sized tufted plant, up to 18 cm in height consisting of numerous stems emerging from the common tangle of rhizoids (a single shoot from Ein Abu Natekina). The more common variant is a medium– or large–sized shoot herbarized alone or as a bunch of partially entangled stems (Figs S1, S2). Their lowest parts are obviously decaying. An enlargement of the lowest stem node with decayed branchlets was detected only once. The lowest decaying parts of the stems are absent in case of the most elongated plants from Solomon's pools (Fig S1b), obviously due to their cutting during collection. The absolute size of apical parts with non–decaying branchlets is different between studied localities with the lowest values observed in Ein Yirka and Ein Shalala and the highest ones in Solomon's pools. The maximal size of available fragments is 64.5 cm recorded in oxbow lake and 75.5–99 cm in Solomon's pools. In all cases, the branching is moderate or, more frequently, scarce.

Our specimens from the quarry are most similar to the holotype by habit (Fig. S1a, b). The plants from other localities are slightly (Ein Abu Natekina) or moderately (oxbow lake, Ein Yirka, Ein Abu Natekina) to strongly elongated (Solomon's pools, Ein Shalala) due to long internodes. The obviously suppressed plants from Ein Abu Natekina, having characteristics of a juvenile stage over much of the stem, are moderately elongated and have slender stems.

The stems are mostly stout, from 435–793(858) to 1500 μm in diameter. The internodes in apical parts are up to 2–times shorter than branchlets or several times longer in strongly elongated plants and usually longer than branchlets within other parts of thallus (Figs 1a, S1, S2). In quarry plants that are not elongated (Fig. S1b), internodes are 0.6–1.9–times longer than branchlets; in moderately elongated plants internodes are 0.6–3.2(6.4)–times longer than branchlets, and 0.6–6.3–times in greatly elongated plants from Solomon's pools (Fig. S1d). The upper parts of non–suppressed thalli looked like spherical loose heads due to short upper internodes and dense long bract–cells (Figs 1a,

S1, S2). The internode length varies between 1.04–18.8 cm with the maximal values from oxbow lake and Solomon's pools.

The stem cortex is diplostichous, moderately or slightly but distinctly tylacanthous, sometimes isostichous (Fig. 1c, d). The latter variant is formed mainly within the older parts. The secondary tube cells usually meet obliquely, very rarely squarely, slightly or sometimes broadly overriding and forming long narrow strips of regular triplostichous cortex. The regular, slightly tylacanthous triplostichous cortex is rarely formed. Solitary intercalary cells between ends of the secondary tube cells were sometimes formed (Fig. 1c). A diplostichous cortex prevails but diplo–triplostichous patches and triplostichous cortex occur.

The stem spine–cells are solitary only, squarrose at right angles to the stem, acute, with distinctly thickened cell walls at their ends, variable in length from short conical–papillose about 1.8–times longer than their width, mainly in the middle and lowest parts of the thallus, conical to long subulate up to stem diameter and to 1.2–2.5–times longer at the upper parts (Fig. 1b–d). The length of spine–cells strongly varies within the whole plant and even within the same internode. The spine–cells are short at elongated internodes. The spine–cells are sometimes deciduous in the lowest and middle parts of the thallus.

The stipulodes are in two tiers, two sets per branchlet, long aculeiform with acute ends, similar with cortex spine–cells, from long, nearly equal to the length of lowest corticated segment of the branchlet to comparatively short (nearly one–fourth– part of the lowest corticated segment) in most cases (Fig. 1e). The length of stipulodes is variable within the same thallus.

The branchlets are usually straight, slightly arcuate or very rarely reflexed, 9–11 in a whorl, 1.6–3.9(4.2) cm in length, 300–510 μm in diameter (Figs 1a, S1, S2). In a single case only the branchlets in the same whorl are unequal to each other, and the difference is nearly 10%. The longest branchlets are found in specimens from oxbow lake (Fig. S1c).

The branchlet consists of 0–1–6 completely corticated segments and ecorticate segment of variable length and consists of variable number of cells being in inverse relationships with the corticated segment's number. Complete or nearly complete branchlets in non–suppressed plants are differentiated, i.e., have regularly formed nodes, which could be easily revealed by the presence of conspicuous bract–cells. If there are 6 corticated segments, the ecorticate segment is a single short conical cell, surrounded with up to 2–times longer, nearly equal or up to 2–times shorter subulate anterior and rudimentary conical posterior bract–cells or two–

celled ecorticate segment that varies from short to equal to the latest corticated segment (Fig. 1f). Rarely, a two-celled ecorticate segment is comparatively long, even slightly longer than the last corticated segment. If there are 4 or 5 corticated segments in a branchlet, the ecorticate segment is a short one-cell or comparatively long two-cell (up to slightly longer than half of the adjoined corticated segment) with or without bract-cells between its cells. Also, with 4 corticated segments, the ecorticated segment consists of 3 and 4 cells. The three-celled ecorticate segment has a single node between lowest cells or two nodes (Fig. 1g). The single corticated segment is basal in most cases, but very rarely it is the second from the base of branchlet.

The branchlet cortex is completely diplostichous but sometimes the tube cells slightly override each other. The incomplete branchlet cortex is formed very rarely on the basal segment of branchlet in a downward direction from its apical node on plants from Ein Abu Natekina only.

The ecorticate segments of non-suppressed plants are nearly all differentiated (Fig. 1f, g). The basal cell of the ecorticate segment is the longest. The cells length within completely ecorticate branchlets gradually decrease to its end. The completely ecorticate branchlets usually consisted of 6 cells; 3 basal nodes are formed within it. The completely ecorticate branchlets could have more unilateral than verticillate bract-cells due to rudimentation of posteriors.

The bract-cells are subulate with distinctly thickened acute ends, distinctly verticillate, up to 4.5 mm in length; posteriors slightly or in 1.8–3-times shorter than anteriors (Figs 1f–i, S2f). The length of bract-cells is variable. The plants from oxbow lake and several specimens from Ein Abu Natekina have usually moderate lengths of bract-cells: from less than one-seventh or one-sixth to one-fifth or one-fourth of the upper corticated segment's length in well developed branchlets up to the half and to the equal length in upper whorls. In other cases, bract-cells are (0.3)0.5–0.8-times shorter (Ein Shalala) and from slightly shorter – slightly-longer to 1.8-times longer (other localities). The maximal relative length of bract-cells characterizes the plants from quarry. The exact differentiation of the specimens originating from different localities on the basis of relative length of bract-cells is nearly impossible due to the overlapping of extreme values of this feature. One extremity of its continual variability refers to the plants from oxbow lake and partially from Ein Abu Natekina; the second one, with maximal values – to the plants from the quarry. The long well developed bract-cells are formed even within greatly elongated thalli from Solomon's pools (Fig. S1d). The lowest length of bract-cells is more characteristic for the lower branchlets. It tend to

decrease from the base to the end of a branchlet.

The gametangia are solitary, conjoined, occurring at the 2–4 lowest nodes between corticated segments (Figs 1h S2f) and between ecorticate segments in a single case only (Fig. 1i). Several oogonia have indistinct neck under coronula. The oogonia are (744) 800–1041 μm in length (excl. coronula) and (418)469–641(700) μm in width. They have (13) 14, 15 convolutions of spiral-cells. The coronula is (103)146 μm in length and 217–297 μm in width. The antheridia are octoscutate, (458)515–583(618) μm in diameter.

The abundance of ripe oospores is low or they are absent in the majority of specimens. A few ripe oospores are found in specimens collected from Ein Shalala, the quarry, and oxbow lake (September collection). The ripe oospores usually have a thick lime-shell. After its removal, they are black in reflective and transmitted light. The oospores have 11–14 striae of moderate height ending at the base of the oospores with basket-like protrusions, which surround the basal plate (Fig. 2a, b). The protrusions are very fragile and are easily destroyed during oospore treatment. The striae end with short claws for these cases. The oospores are 745–881 μm (incl. protrusions) or 652–776 μm (excl. protrusions) in length and (303)343–503 μm in width. The oospore ornamentation varies from nearly smooth and indistinctly pustular through pustular to papillate (Fig. 2c, d), probably depending on the stage of ripening, as could be concluded from the oospores where ornamentation was formed unevenly at different fossa. The ornamentation of fossa and ribs is identical. The disrupted oospore wall is brownish in transmitted light.

The suppressed plants from Ein Abu Natekina are up to 22–24 cm in height. They have diplostichous tylocanthous stem cortex, rudimentary conical-papillose stipulodes, and mostly undifferentiated naked branchlets without bract-cells. In a few cases with upper whorls, only the lowest node of branchlet has rudimentary bract-cells. The 1 or 2 corticated segments are present within apical whorls of branchlets only.

The individuals consisting of a single ramet – ortotropic axis (BOCIAG & REKOWSKA, 2012; BOCIAG et al. 2013) without rhizoids prevailed among the studied specimens. The strong apical dominance, with the main axis and single, usually short, branch at several nodes was their general habit. Probably, in these cases, growth of the apex of *C. globata* is balanced by basal decay of the plant as in *C. hispida* L. and other *Chara* species (ANDREWS et al. 1984). Obviously, different environments might be favorable for the ramets of different sizes expressed as a number of nodes. An individual consisting of several ramets with abundant rhizoids was also present but in a single case only (Ein Abu Natekina).



Fig S1. Habit of herbarium specimens of *C. globata*: (a) paratype (LE), (b) plant from the Ma'agan Michael Quarry (TELA), (c) plant from the oxbow lake (NS), (d) plants from the Solomon's pools (TELA). Scale bar 2 cm.

S2. Description of localities in Western Asia and South–Eastern Europe

A list of localities have been compiled according to the herbarium specimens' labels and supplemented with available information about their geographical locations. The first name of the locality is reported according to labels.

1. Western Asia, Israel, Coastal Plane, Tanenim, Shomron, Ma'agan Michael Quarry, small pool in kurkar quarry, 32°34'01"N 34°55'10"E, 11 m a.s.l., 28.05.1970, Y. LIPKIN (TELA: No 20373, 20374). The inundated quarry in Kibbutz Ma'agan Michael is made up of calcareous sandstone of marine origin forming ridges in the northern coastal plain of Israel (SIVAN & PORAT 2004). It is located near the Mearot River mouth, but never connected with the river because the quarry is about ten meters above the river. This locality was surveyed during May 2014 without success. The bottoms' sediments of the quarry were probably silty because specimens were partly covered with fine light–brownish silt. Based on specimens we checked in TELA, *C. vulgaris* var. *longibracteata* (KÜTZ.) J. GROVES & BULL.–WEBST. also had been collected here by Y. LIPKIN on 04.05.1967. The kurkar quarry still exists. The quarry banks are steep and slippery. The quarry size is about 150 × 100 m with a depth of about 20 m. Water covered only the bottom of the quarry with a depth of no more than 0.5 m. Plastic carpets covered the banks of the quarry for protection from stones falling. The water was brackish with conductivity about 9.75 mS.cm⁻¹ (01.05.2014). The pH values ranged between 7.0 and 7.1. The nitrates concentration in water varied between 0.7 mg.dm⁻³ and 1.9 mg.dm⁻³. A few clumps of halophilous *Ruppia maritima* L. grew in the pond. The charophytes have not been found.

2. Western Asia, Israel, Judean Mountains, east part, south of Bethlehem, Solomon's pools [Berekhot Shelomo, Breichot Shlomo, Birkat as–Sultan Suleiman al–Kanuni], 31°41'19"N 35°10'17"E, 784 m a.s.l., with *Tolypella glomerata* (Desv. in Loisel.) Leonh., 12.06.1969, V. PROCTOR, Y. LIPKIN (TELA: No 2304–2308). The so–called Pools of Solomon, three in number, situated on the south–south–west precincts of Bethlehem, are fed by surface water and by aqueducts from springs. These reservoirs in limestone bedrock were created in antiquity as water–supplying systems, which had been crucial to Jerusalem's water supply from 2 BC to 20 AD (BROMILEY 1995; MURPHY–O'CONNOR 2008). N. BOVÉ (1834) made the first charophyte collection here in the 19th century. He found *Chara* on July 23, 1832. From these specimens, A. BRAUN described *C. fragilis* Desv. var. *meridionalis* A. Braun (*C. meridionalis* (A. BRAUN) KÜTZ.; BRAUN 1834, 1835; DECAISNE 1834; KÜTZING 1849; WALLMAN 1854, 1856). Also, this taxon was reported from this habitat

as *C. vulgaris* without author citation (THOMSON & THOMSON 1835). E.M. BLACKWELL found monospecific communities of *Chara* in Solomon's pools in August 1923 after a significant water–level decrease; *C. connivens* SALZM. ex A. BRAUN was dominant in the lowest pool (WASHBOURN & JONES 1937). *C. connivens* and *C. globularis* Thuill. were reported by V. PROCTOR (1971) based on a collection of Y. LIPKIN. Based on specimens we checked in TELA, *C. connivens*, *C. contraria* A. Braun, and *C. vulgaris* var. *longibracteata* were collected from these reservoirs by Y. LIPKIN on 31.07.1967 and *C. contraria*, *C. globularis* – by Y. LIPKIN on 03.10.1967.

3. Western Asia, Egypt, Central Sinai, northern from northwestern part of Tih plateau, eastern (upper) part of Wadi el Soq, spring pools Ein Shalala [Ain Shelala, Shelalla, Ain el Shallala, En Ashala], ~29°28'24"N 33°12'58"E, ~500 m a.s.l., 07.01.1970, E. COHEN (TELA: No 20343–20345). The upper part of Wadi Wardan – Wadi Shelala is cut out in the Cenomanian series, which is mostly composed of soft, sandy, and clayey beds but includes bands of intensely hard limestone. A small stream in Wadi Shelala originating from perennial springs forms a cascade with deep pools of clear water below (BEADNELL 1926). This is a source of drinking water. The chlorine ion concentration in waters of Ein Shalala (reported as En Ashala) was 825 mg.dm⁻³ 03.1969 and 20.08.1969 (MARGALIT & TAHORI 1973).

4. Western Asia, Egypt, Central Sinai, northern from northwestern part of Tih plateau, drainage basin of Wadi el El Arish, spring Ein Abu Natekina [Ain Abu Nataigina, Ain Abu en Natvikana, En Abu Natagna, Ain Abu en Nateykana, Ain Abu el Nateiqana], 29°16'N 33°31'E, ~700 m a.s.l., with *C. contraria* and *C. vulgaris*, 03.01.1970, E. COHEN (TELA: No 20338–20342).

5. Western Asia, Egypt, Central Sinai, northern from northwestern part of Tih plateau, southern (upper) stretch of Wadi Yarqa, spring pool Ein Yirka [Moyat Yerga, Moya't Yerga, 'Ain Yarqa, En Yarqa, Ayn–Berga, Ayn–Verga], within 29°20'18.6–21.9"N 33°28'23.3–25.8"E, ~700 m a.s.l., with *C. contraria* and *C. vulgaris* var. *longibracteata*, 05.01.1970, E. COHEN (TELA: No 20330–20333). Occasional heavy rains which fall on the southern uplands of the Tih plateau are largely absorbed by the Turonian limestones, filtering through the crevasses to reappear as springs at the lower levels in the north. Ein Yirka and Ein Abu Natekina are sources of drinking water. They are situated within the southwestern part of the Wadi el Arish drainage basin (BEADNELL 1926) – the largest ephemeral drainage system of Sinai (ABUBAKR et al. 2013). The Ein Yirka is a wellspring deep permanent pool at the head of a narrow gorge. It harbors good clear

drinking water uncontaminated by livestock, which can only access the tail-end of the pool (BEADNELL 1926). In actual Google maps a series of at least five pools can be found with sizes not more than 40×2.9 m in the central pool and 25×7 m in the upper pool. The chlorine ion concentration in waters of Ein Yirka, Ein Abu Natekina, and Bir Malha (reported as En Yarqa, En Abu Natagna, and Bir Malha) was 200–400 mg.dm⁻³ 02.08.1969 (MARGALIT & TAHORI 1973).

6. Eastern Europe, Russian Federation, Krasnodar Region, Kushchyovsky District, stanitsa Shkurinskaya (46°35'N, 39°21'E), oxbow lake of the Yeya River, monospecific stands, 26.06.2013, 12.09.2013, A.N. LUBCHENKO (NS: No 500–507).

The Yeya River flows in Krasnodar Krai of the Russian Federation, which is historically known as Kuban. It empties into the Yeya Firth of the Taganrog Bay, Sea of Azov. Its drainage basin is situated at steppe zone and nearly all consist of arable lands. The Yeya River is a lowland river with a length of 311 km, a drainage basin of 8.6 10³ km², and a low mean annual discharge not exceeding 2.5 m³.s⁻¹ (BORISOV 1978). The river water has comparatively high hardness and salinity with increasing total dissolved solids' concentration from 3 g.dm⁻³ to 8 g.dm⁻³ down the river. The river waters at low-water periods mainly belong to the second type of sodium sulfate waters according to classification of ALEKIN (1970), i.e., the sulfate and sodium ions predominate, and the concentration of bicarbonate ion is less than the concentrations of calcium and magnesium ions (BORISOV 1978). The unnamed oxbow lake is situated in the middle stretch of the Yeya River. *Chara globata* formed mono-specific stands with a projective cover of 100%, nearly reaching the surface of the water.

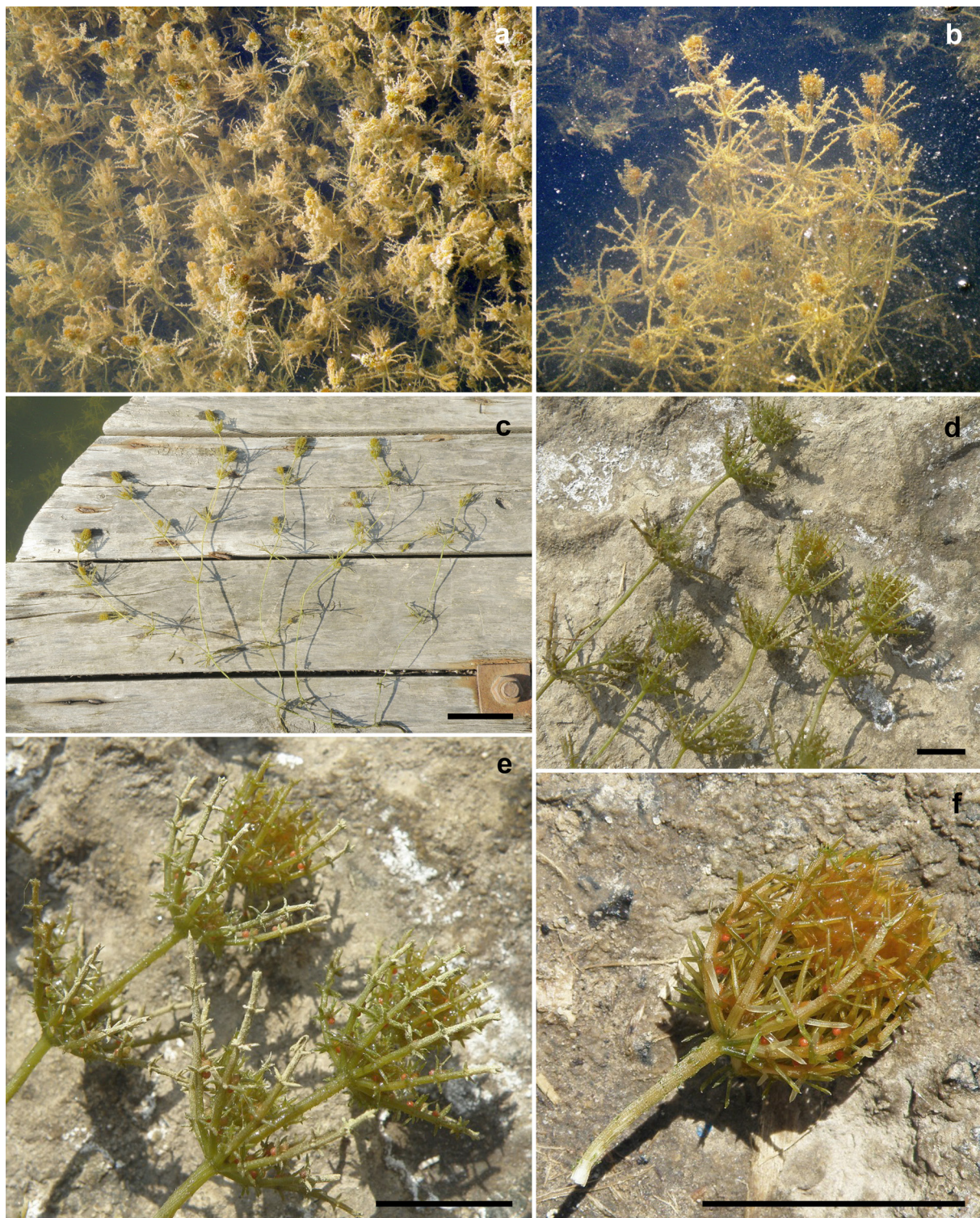


Fig S2. Habit of live specimens of *C. globata* from the oxbow lake. Photos of A.N. LUBCHENKO. Scale bar 2 cm, (c) 10 cm.

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