Chara globata Mig. (Streptophyta: Charales): rare species revised

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Abstract: A rare species *Chara globata* Mig. has a distinct habit and may be referred to as a flagship species. Here we describe a wide range of vegetative and reproductive morphologies in *C. globata* in localities distant from the *locus classicus*. In contrast to the known holotype features, the specimens studied consistently displayed a generally tylacanthous diplostichous stem cortex which is in agreement with protologue. Based on morphology, phylogenetic affinity (this study) and results of breeding experiments (Proctor 1971, 1975), we proposed that *C. globata* should be transferred to the section *Chara*. Current data suggest that *C. globata* is a species with a disjunctive distribution, mainly in the arid and semiarid regions of Eurasia. Specimens collected from the end of the 1960s to the beginning of the 1970s confirmed the existence *C. globata* in Western Asia. The species was also recorded in Europe and the Sahara–Arabian Desert for the first time.

Key worlds: *Chara globata*, charophytes, distribution, ecology, morphology, oospores, phylogeny, taxonomy

INTRODUCTION

Eighty–one extant macrospecies and nearly 395 microspecies of charophytes that have been described to date are classified according to the morphospecies concept (Wood & Imahori 1965). Although some of these microspecies are frequently listed in regional checklists and floras, many others are rarely mentioned in the literature. This may reflect an absence of the species in the region studied, as well as their misidentification or unrepresentative sampling. A few large– and medium–sized charophytes have distinct habits, which makes it almost impossible to overlook these species within the charophyte collection, meaning that these species could be referred to as flagship species: "Flagships are so showy, or so novel, making it unlikely that they would be overlooked" (Foissner et al. 2008: 117). This concept is a useful tool for the biogeography of protists and microalgae (Tyler 1996; Foissner 2006) and can probably be applied to other groups of living organisms. The scarcity of records for flagship species likely reflects their extreme rarity or probable absence in the extensively studied regions. *Chara globata* Mig. is one of such a species, having distinct habit, resembling *Lychnothamnus* (Migula 1904; Wood & Imahori 1964, 1965) or *Chara vertillibracteata* Y.J. Wang et W.T. Su in W.T. Su et al. (Han & Li 1994).

Chara globata has been rarely reported since its initial description (Migula 1904; Wood 1952, 1959, 1962, 1964; Wood & Imahori 1959, 1964, 1965; Bicudo 1976; Soulie–Märsche 1989). It is known from eastern, northwestern and southwestern China (Migula 1904; Han & Li 1994; Deng & Zhang 1996; Ling et al. 2000), southern Kazakhstan (Kostin & Shoyakubov 1972 1973a,b, 1974a,b; Shoyakubov 1973, 1979) and Israel (Milner & Avigad 1972; Brimacombe et al. 1973). *C. globata* was used in breeding experiments for taxonomic purposes and for tests of the applicability of the biological species concept to charophytes (Proctor 1971, 1975).

Morphological descriptions and illustrations of *C. globata* are based on the re–examined holotype only. Phenotypic characteristic of *C. globata* remain incomplete due to limited information about oospore morphology and range of morphological variability. The morphological descriptions, distributional records, and habitat characteristics of *C. globata* are scarce and require supplementation and improvement. *C. globata* has been assigned to the section *Grovesia* R.D. Wood, subsection *Grovesia* R.D. Wood (Wood 1962, 1964; Wood & Imahori 1965) and section *Diplostephanae*, series or subsection *Triplostichae* (Han & Li 1994; Ling et al. 2000). However, these placements were not supported by results of breeding experiments, that instead pointed towards its affinity with members of section *Chara*, subsection *Hartmania* R.D. Wood (Proctor 1971, 1975). An improvement in *C. globata* description is needed for clarification of its taxonomic and phylogenetic affinities within the genus, as for many charophyte species.

Here we aimed to study the morphology and molecular phylogenetic affinity of rare species *C. globata* based on the specimens collected in localities distant from *locus classicus*.

Materials and Methods

Preparations and study of specimens. Nineteen herbarium sheets labeled as *Chara globata* were found during examination of the Israeli charophytes collection stored in Tel Aviv University Herbarium – TELA (Romanov & Barinova 2012). The specimens were collected by E. Cohen, Y. Lipkin, and W. Proctor in 1969 and 1970 in Israel and Egypt. Recently, A.N. Lubchenko revealed a population of *C. globata* on the southern European part of Russia and kindly sampled it at our request. These specimens were deposited in the NS (Herbarium of Central Siberian Botanical Garden SB RAS). Altogether, 26 herbarium sheets were examined.

Specimens were treated according to Hollerbach & Krasavina (1983). The photos were taken with a Carl Zeiss Stereo Discovery V12 stereomicroscope equipped with an AxioCam MRs–5 digital camera. The terms "differentiated" and "undifferentiated" segments were used for the branchlet part with and without nodes (Frame 1974). Consequently, the formation of a cortex and bract–cells or only bract–cells has been assumed as indicators of the formation of nodes within branchlets.

The oospores were treated with acetic acid to remove any lime–shell, washed with distilled water and cleaned from spiral cells by adding 10% Triton X100, then stored at 60 °C for at least 10 hours. Finally, they were washed with distilled water and sonicated to remove spiral cells completely. The cleaned oospores were stored in 95% alcohol. They were coated with zinc and studied with a scanning electron microscope ZEISS EVO 40 (Carl Zeiss) at 17.54 kV. The terms used for describing the oospore surface followed those from Urbaniak (2011). All photos were digitally manipulated and plates were created using Adobe Photoshop 8.0®.

The DNA was extracted with the modified Echt's method (Echt et al. 1992; Kiselev et al. 2011) from the specimens collected in the Maagan Michael Quarry (Israel) and oxbow lake of the Yeya River (Russia), where the sizes of specimens were allowed to detach from several apical parts of thalli without obvious deterioration. A partial rbcL gene was amplified with primers RH1 and 1385R (Manhart et al. 1994), and PCR products were directly sequenced using the ABI Big Dye Terminator v.3.1 Cycle Sequencing kit according to the manufacturer's instructions and the ABI 3130 genetic analyzer at the Institute of Biology and Soil Science FEB RAS. The resulting sequences were submitted to the NCBI nucleotide database with the following accession numbers: LM653113 (TELA: 20373) and LM653114 (NS: 500).

To assess the phylogenetic relationship of *C. globata* we assembled a data set including putatively related *C. hispida* (L.) Hartm. (HF912657), *C. intermedia* A. Braun (HF912656), *C. rudis* A. Braun in Leonh. (HF912655), *C. baltica* Bruzelius (HF912653), *C. polyacantha* A. Braun in A. Braun, Rabenh. et Stizenb. (AY170453), *C. contraria* A. Braun ex Kütz. (HF912659), *C. tomentosa* L. (HF912646), *C. vulgaris* L. (DQ229107), *C. aspera* Willd. (HF912645), *C. connivens* Salzm. ex A. Braun (HF912648, AF097161, L13476, AF097162), *C. virgata* Kütz. (HF912649), *C. globularis* Thuill. (AB440244, AF097163, HF912647, F097164), *C. canescens* Desv. et Loisel. in Loisel. (AM710330), *C. fibrosa* C. Agardh ex Bruzelius (AB359168), *C. zeylanica* Klein ex Willd. em. R.D. Wood (AB359169), *C. rusbyana* M. Howe (AF097168), *C. corallina* Klein ex Willd. em. R.D. Wood (AB359167), and *C. braunii* C.C.Gmel. (AB363843) in SeaView (Galtier et al. 1996) and analyzed with Maximum Parsimony method in PAUP* 4.0b10 (Swofford 2002) by a heuristic search with tree–bisection–reconnection (TBR) branch swapping and 10 random sequence addition replicates. The support for branches was calculated by bootstrap analyses with 1000 replications of heuristic search.

Results and Discussion

Morphology
Existing morphological descriptions

Chara globata Mig.

(Migula 1904: 537, 538; Wood & Imahori 1964: Icone 87; Wood & Imahori 1965: 213; Han & Li 1994: 235, 236, fig. 182; Ling et al. 2000: 87)

C. globata was initially described as a monoecious diplostephanous diplostichous slightly tylacanthous species with corticated branchlets with 1 to 2–celled ecorticate segments, and conjoined gametangia; and occupying a distinct habit due to its peculiar clenched–like whorls of branchlets with long bract–cells and bracteoles (Migula 1904). It should be emphasized that stem cortex arrangement according to protologue (Migula 1904) contrasts the later description of holotype (Wood & Imahori 1964, 1965; Han & Li 1994), reporting irregular (haplo–diplo)–triplostichous isostichous cortex. This discrepancy may reflect the differences in the studied material with the description by Wood & Imahori (1964, 1965) being based on a single broken specimen consisting of four fertile heads and one main axis. Unfortunately the type specimen was lost and the only currently available paratype is stored in LE (Fig. S1a). These paratype specimens have a slightly tylacanthous and isostichous stem cortex.

The description in the "Flora Algarum Sinicarum Aquae–Dulcis" repeats the holotype description (cf. Wood & Imahori 1965; Han & Li 1994). It could be that specimens collected in China from localities other than *locus classicus* fitted the existing description, or that they were not described. Later Chinese specimens of *C. globata* were described as monoecious with irregular triplostichous tylacanthous cortex, solitary spine cells, developed stipulodes, 6 branchlet segments, 5–6 developed bract–cells, and conjoined gametangia (Ling et al. 2000).

Morphological description of studied specimens

The degree of incrustation varies in specimens from different localities (for details see S1); from calcite–unencrusted with slightly encrusted old parts to the moderately and heavily incrusted. 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. The more common variant is a medium– or large–sized shoot herbarized alone (Fig. S1) or as a bunch of partially entangled stems, with the lowest parts decaying. The maximal size of available fragments is 64.5 cm recorded in oxbow lake and 75.5 to 99 cm in Solomon's pools.

The stems are mostly stout, from 435–793(858) to 1500 µm in diameter. The internodes in the apical parts are up to 2–times shorter than branchlets or several times longer in strongly elongated plants, and usually longer than the branchlets within other parts of the thallus. The upper parts of non–suppressed thalli look like spherical loose heads due to their short upper internodes and dense long bract–cells (Figs 1a, S1a–c).

The stem cortex is diplostichous, moderately or slightly but distinctly tylacanthous, sometimes isostichous (Fig. 1b–d). The regular, slightly tylacanthous triplostichous cortex is rarely formed. Although a diplostichous cortex prevails, diplo–triplostichous patches and triplostichous cortexes do occur. The stem spine–cells are solitary only, squarrose at right angles to the stem, acute, with distinctly thickened cell walls at their ends, and variable in length from short conical–papillose to conical and long subulate (Fig. 1b–d).

The stipulodes are in two tiers two sets per branchlet, and long aculeiform with acute ends (Fig. 1e). 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, and 300–510 µm in diameter (Fig. 1a, f). The branchlet consists of 0–1–6 completely corticated segments and with an ecorticate segment of variable length that consists of a variable number of cells (having an inverse relationships with the corticated segment's number). The branchlets in non–suppressed plants are completely or nearly completely differentiated (i.e., have regularly formed nodes), which could be easily revealed by the presence of conspicuous bract–cells (Fig. 1g). The branchlet cortex is completely diplostichous.

The bract–cells are subulate with distinctly thickened acute ends, distinctly verticillate, up to 4.5 mm in length, and slightly posteriors or 1.8–3–times shorter than the anteriors (Fig. 1a, f–i). The length of the bract–cells is variable. The exact differentiation of the specimens originating from different localities on the basis of the relative lengths of their bract–cells is almost impossible due to the overlapping of extreme values of this feature.

The gametangia are solitary, conjoined, occurring at the 2–4 lowest nodes between corticated segments (Fig. 1h) and between ecorticate segments in a single case only (Fig. 1i). The antheridia are octoscutate.

The abundance of ripe oospores is low or they are absent in the majority of specimens. The ripe oospores 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 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). The ornamentation of the fossa and ribs are identical.

Comparison of existing morphological descriptions

The stem cortex arrangement of studied specimens is in agreement with the protologue of *C. globata* (Migula 1904) and description of other Chinese specimens (Ling et al. 2000). Our specimens differed from the diagnosis based on holotype re–examination (Wood & Imahori 1964, 1965) in the following features:

1) Generally, a tylacanthous diplostichous cortex, but strips or patches of irregular cortex occurred in contrast with irregular (haplo–diplo)–triplostichous isostichous cortexes of the holotype. This is the most significant difference.
2) Usually has a greater number of branchlet segment including cells of the ecorticate segment, as in an individual segments described by R.D. Wood & Imahori (7–8) and the corticated segments (5, 6) within it; their numbers sometimes correspond to those indicated in the diagnosis (6 and 4) or less (5 and 0–3);
3) More variable length of the end cell of a branchlet;
4) Greater number of branchlets in a whorl: 9–11 vs. 6–8(11?);
5) Higher maximal absolute and relative values of internode length (up to 18.8 cm and 6.3–times longer than the branchlet);
6) Wider range of absolute and relative lengths of spine cells;
7) Presence of ecorticate branchlets with developed bract–cells at several basal nodes;
8) Sometimes longer ecorticate segments;
9) The bract–cells can be smaller than in the holotype although they are fully developed and never rudimentary. This feature provides for a habitual similarity with *Lychnothamnus*, but a *Lychnothamnus*–like habit would be unusual for all populations of *C. globata*;
10) Higher maximal length of branchlets;
11) Variable degree of thallus incrustation, from almost un–incrusted to heavily incrusted;
12) Fewer number of oospore striae: 11–14 vs. 15;
13) Basket–like protrusion formation on the basal pole of oospores.

The quantitative differences listed above (No 3–5, 7–10) haven`t hiatus with the holotype description and don't have primary taxonomical importance at the species level. Moreover, some of these differences are known to be environmentally–dependent. In particular, the variable absolute and relative lengths of internodes and branchlets may reflect light intensity (Schneider et al. 2006), while the degree of incrustation can reflect plant age, photosynthetic activity, calcium and carbonate ion concentrations, and pH and temperature of the habitat (Smith 1985, cited by Kawahata et al. 2013).

The differences in taxonomically important characteristic (No. 1) and presence of previously undescribed (No. 6, 12) or poorly described features (No. 11) could be explained by the individual peculiarities of the holotype, including absence of ripe oospores. The discrepancy in cortex arrangement was actually quantitative because the patches of the diplo–triplostichous cortex were also presented in studied specimens; therefore, this difference was expressed in frequency of different patterns occurrence.

Another possible explanation of this inconsistency of stem cortex arrangement in a different distribution range is phenomenon that is well–known for *C. tomentosa* L.; where different specimens have non–identical expression of this characteristic. They usually have a diplostichous cortex but specimens with a triplostichous cortex are also known (Olsen 1944; Torn et al. 2003). Sometimes different cortex patterns may be observed in specimens from the same lake collected in different years (Romanov & Kipriyanova 2010).

The specimens we studied better fitted the protologue (Migula 1904) than the later re–description (Wood & Imahori 1964, 1965) in several features (i.e., common type of stem cortex arrangement and number of branchlets in a whorl) but differed from protologue and re–descriptions, both in the usually greater number of branchlet segment and corticated segments within it. The incomplete matching of other characteristics within the previous description might be regarded as intraspecific variability better represented in the studied collections. The oospore surface ornamentation had never been described for this species. Therefore, our description based on specimens outside of the *locus classicus* expands and improves the diagnosis of *C. globata*.

Systematics

C. globata was assigned to the section *Grovesia* R.D. Wood, subsection *Grovesia* R.D. Wood (Wood 1962, 1964; Wood & Imahori 1965) and section *Diplostephanae*, series or subsection *Triplostichae* (Han & Li 1994; Ling et al. 2000). The macrospecies concept implemented by Wood in the taxonomic treatment of charophytes significantly reduced the number of species due to assigning many described species to varieties, forms, and synonyms (Wood 1964; Wood & Imahori 1965). Nevertheless, the species status of *C. globata* was retained as a reflection of the prominent combinations of its characteristics (Wood & Imahori 1965: 213).

The crossing experiments seemingly confirmed the sexual incompatibility of *C. globularis*–complex members (section *Grovesia*, subsection *Grovesia*) with *C. zeylanica* Willd. (section section *Grovesia*, subsection *Willdenowia*), *C. vulgaris*, *C. contraria* (section *Chara*, subsection *Chara*), and *C. globata* (Proctor 1971, 1975). In contrast, *C. globata* from Israel as well as a plant resembling *C. domini* Vilh. from Uzbekistan, crossed freely and reciprocally with plants of *C. polyacantha* A. Braun in A. Braun, Rabenh. et Stizenb. from Denmark. Hybrid offspring were vegetatively near normal but often wholly or partially self–sterile. Unfortunately, the collection sites for these specimens were not published, however, according to the context and available data from labels of specimens stored in NY (http://sciweb.nybg.org/Science2/vii2.asp), we may conclude that specimens from the quarry, Solomon's pools, and one locality from Central Sinai, had been used.

The mostly diplostichous stem cortex typical for studied specimens of *C. globata* is in contrast with a main feature of species from the section *Growesia*, having a triplostichous cortex. Obviously, the assignment of *C. globata* to this section may reflect peculiarities of the holotype, which have been discussed above. The morphological characters as well as negative results of breeding experiments point towards erroneous placement of *C. globata* within this section. The cortex arrangement, solitary spine–cells only, and stipulodes in two tiers, all correspond well to the section *Chara*, subsection *Chara* sensu Wood (Wood 1962; Wood & Imahori 1965). However, this was not supported by crossing experiments, which pointed toward an affinity of *C. globata* with species from subsection *Hartmania* (Proctor 1971, 1975). Therefore, according to morphological characteristics and sexual compatibility, *C. globata* should be transferred to section *Chara*. The subsection placement is problematic and needs further investigation with a better taxon sampling and likely additional molecular markers.

Phylogenetic affinity

Two *C. globata* accessions (Israel and Russia) have produced identical *rbc*L sequences. Based on a data set of 1.072 aligned characters (with 26 potentially parsimony–informative characters), *C. globata* was resolved in a moderately supported clade (82%) comprising members of the section *Chara* and subsections *Hartmania* and *Chara* (*C. contraria*). There it was placed as a sister (90% bootstrap, only 2 positions difference) to five species of sub–section *Hartmania* having identical sequences (Fig. 3). *C.*

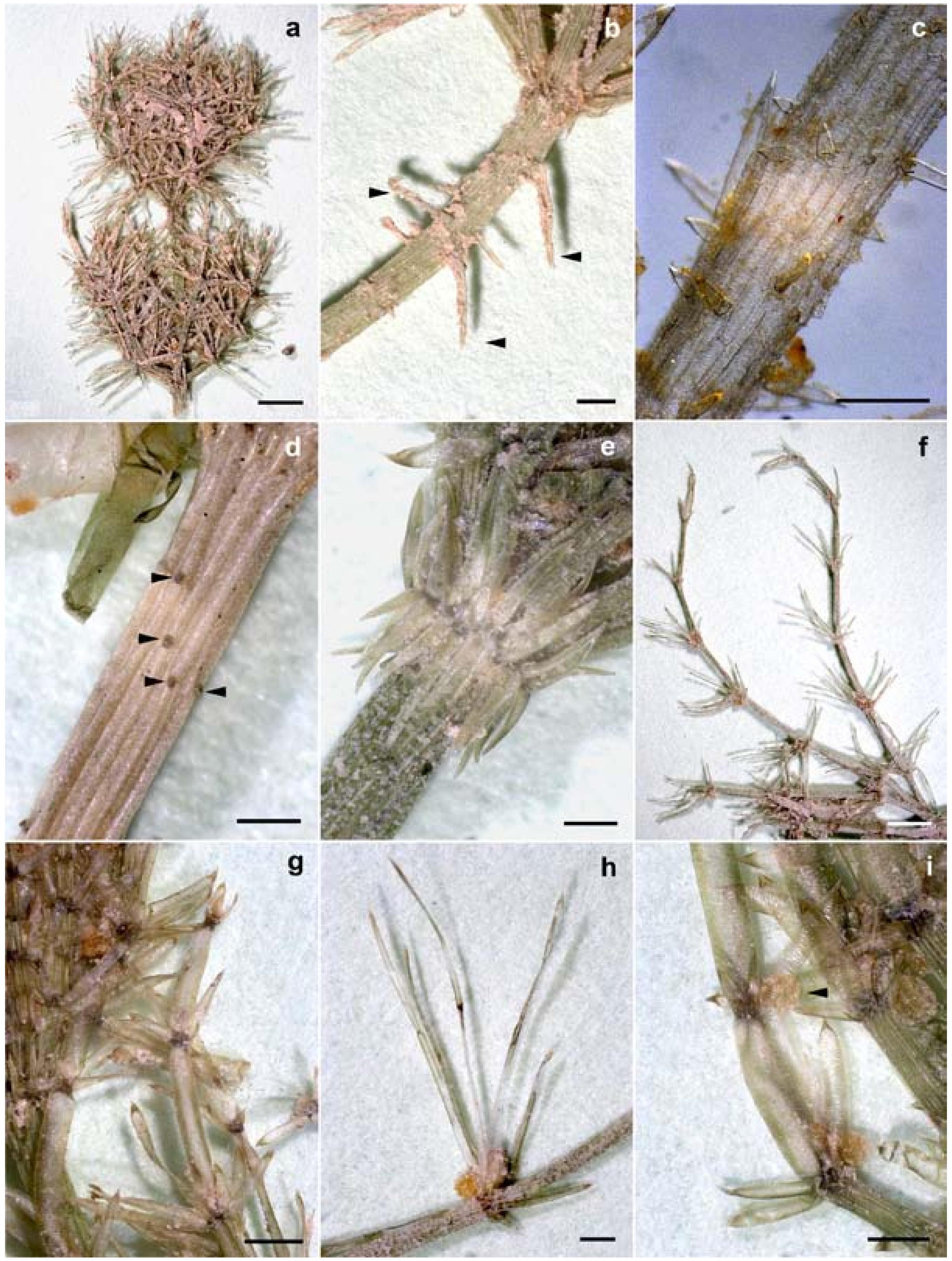

Fig. 1. Morphological characters of *C. globata*, LM: (a) whorl of branchlets; (b–d) stem cortex (arrowheads indicate spine cells); (e) stipulodes; (f) branchlet; (g) ecorticate segment of branchlet; (h) node of branchlet between corticated segments with bract cells; (i) ecorticate fructified segment of branchlet (arrowhead). Scale bar 0.5 mm, exept (a) 2 mm.

Table 1. The differential characters and distribution of species similar with *C. globata*

Species	Differential characters from *C. globata*	Distribution	References
Chara domini Vilh.	rare geminate spine–cells; bigger diameter of antheridium (Table 2), shorter branchlets and bract–cells	mainly Central Asia: Southern Kazakhstan, Uzbekistan, and Turkmenistan, two localities in Ukrainian Polissia and in Ukrainian Steppe	Vilhelm 1928; Shoyakubov 1979; Hollerbach & Krasavina 1983; Hollerbach & Palamar–Mordvintseva 1991; Barinova et al. 2014
C. tenuispina A. Braun	relatively small–sized; regular triplostichous isostichous stem cortex; occasionally with a slightly irregular arrangement with 1–3 secondary tube cells; triplostichous branchlet cortex	mainly Europe, few localities in Northern Kazakhstan, Uzbekistan, and China	Wood & Imahori 1965; Shoyakubov 1979; Han & Li 1994; Gąbka 2007; Krause 1997 Sviridenko & Sviridenko 2003
C. vertillibracteata Y.J. Wang et W.T. Su in W.T. Su et al.	isostichous diplostichous cortex and papillose spine–cells; shorter stipulodes; shorter and relatively wider oogonia and oospores	Eastern China	Han & Li 1994; Ling et al. 2000
C. tomentosa L.	dioecious; relatively short spine–cells, solitary and clustered of 2–3; swollen bract–cells, cells of branchlet ecorticate segment and spine–cells	Europe, Northern Africa, Western and Central Asia, Southern Ural, Southern Siberia, Mongolia, and North–Western China	Hollerbach 1950; Wood & Imahori 1965; Shoyakubov 1979; Kostin 1987; Han & Li 1994; Hollerbach & Krasavina 1983; Krause 1997; Torn et al. 2003; Veisberg & Isakova 2010; Romanov & Kipriyanova 2010; Ahmadi et al. 2012; Romanov et al. 2014

Notes: The spine–cells arrangement was not described in the protologue of *C. domini* (Vilhelm 1928). The holotype and other specimens from Central Asia were studied by Hollerbach and Krasavina (1983). The spine–cells were described as well–developed, solitary or rarely geminate mainly relatively long, ½–¾ of stem diameter, rarely slightly longer in stem diameter, acute or nearly papillose.
The European specimens of *C. tenuispina* have shorter oogonia and oospores, smaller diameter of antheridia (Table 2); the maximal values of oogonium and oospores length are overlapping with minimal values for *C. globata* and *C. domini*. The Chinese specimens of *C. tenuispina* do not differ exactly by these characteristics.

Table 2. The quantitative characteristics of gametangia and oospores of *C. globata*, *C. domini*, *C. tenuispina* and *C. vertillibracteata* (^{a}Migula 1904; ^{b}Wood & Imahori 1964, 1965; ^{c}Shoyakubov 1979; ^{d}Hollerbach & Krasavina 1983; ^{e}Han & Li 1994; ^{f}Krause 1997; ^{g}this study).

Characteristics	*Chara globata*	*C. domini*	*C. tenuispina*	*C. vertillibracteata*
Length of oogonium (excl. coronula) (µm)	~1000^{a} 750 (immature)–900^{b} (744) 800–1041^{g}	1105–1158^{c} 1000–1200^{d}	620–1200^{e} 500–600–(800)^{f}	580–600^{e}
Width of oogonium (µm)	550–600^{a} 570 (immature)–645^{b} (418)469–641^{g}	600–686^{c} 500–700^{d}	320–560^{e} 400–500^{f}	450–500^{e}
Number of convolutions of spiral cells	16–18^{a} >10^{b} (13) 14, 15^{g}	13–15^{c} 13–15^{d}	12–15^{e} 12–15^{f}	14–15^{e}
Length of oogonium coronula (µm)	120^{a} (120?)345–375^{b}	72–129^{c} 130–205^{d}	105–120^{e} 100–150^{f}	100–150^{e}
Width of oogonium coronula (µm)	160^{a} 180–195^{b}	200–286^{c} 285–320^{d}	180–230^{e} 150–200^{f}	200–250^{e}
Diameter of antheridium (µm)	320^{a} (320)450–525^{b} (458)515–583(618)^{g}	unripe 372–472^{c} 640–760^{d}	300–520^{e} 250–300^{f}	450–500^{e}
Length of oospore (µm)	~800^{a} c. 800^{b} 745–881 or 652–776^{g}	772–815^{c} (772)850–920^{d}	500–800^{e} 450–650^{f}	450–460^{e}
Width of oospore (µm)	400^{a} 400^{b} (303)343–503^{g}	386–457^{c} 386–470^{d}	230–300^{e} 300–450^{f}	350–360^{e}
Number of oospore ridges	15^{a} 11–14^{g}	11–14^{c} 13–14^{d}	11–13^{e} 11–14^{f}	13–14^{e}

vulgaris and *C. tomentosa,* also members of the subsection *Chara*, showed only a distant relationship to *C. globata* and this lineage.

The results of our phylogenetic analysis generally corroborate conclusions based on phenotypic data and suggested that *C. globata* has no affinity to the section *Growesia*. Rather, the species shows a close relationship to the subsection *Hartmania* and to some members of the sub–section *Chara* (*C. contraria*), although not monophyletic in our analysis.

Similar species

C. domini Vilh. (the name follows protologue (Vilhelm 1928), *C. tenuispina* A. Braun, *C. vertillibracteata* Y.J. Wang et W.T. Su in W.T. Su et al., and *C. tomentosa* are similar to *C. globata* in combinations of characteristics or general habit. We compared the description of ranges of these species and distribution range to outline the borders and distinctive characters of species similar to *C. globata* (Table 1). The other characteristics may be easily compared using a matrix key to the charophytes species (van Raam 2009). By this approach *C. domini* is the most similar species. The specimens referred to as *C. domini* from Uzbekistan (Shoyakubov 1979) closely resembled the studied specimens of *C. globata*. Plants from Uzbekistan differed by bushy habit, regular diplostichous tylacanthous stem cortex only, fewer branchlets in a whorl (7), higher upper values of the number of corticate segments (7), length and width of oogonia (1158 and 686 µm), length of oospore (815 µm), and thick lime–shell of oospore. Therefore, these specimens were more similar to *C. globata* than *C. domini*, and possibly with several other records of *C. domini*. Therefore, *C. globata* is possibly conspecific with *C. domini* and in this case the first name would have priority but this conclusion should be validated by study of numerous specimens of C. *domini,* including the type specimens.

Distribution of *C. globata*

C. globata was first described from Beijing (Peking) based on a collection of E. Bretschneider made in 1877 (LE; Migula 1904; Wood & Imahori 1965). The characteristics of the *locus classicus* are unknown, including the type of water body (LE; Migula 1904). The collections of Bretschneider were mostly taken from the northwest vicinity of Beijing and within the city (Bretschneider 1898).

C. globata was reported for China not only from *locus classicus* but also from arid and semiarid regions (i.e., from one locality in Gansu and three localities in Xinjiang Uyghur Autonomous Region; Han & Li 1994; Ling et al. 2000). It is also known from Yunnan (Deng & Zhang 1996). We believe this species also be listed in several other inaccessible Chinese articles. The recent photo of a specimen very similar to *C. globata* was posted at a forum on September 25, 2009 (http://www.planta.cn/forum/viewtopic.php?t=18248&view=previous; Image DSCF1475. JPG). It had been collected within Eryuan County (Ěryuán Xiàn) in the Dali Bai Autonomous Prefecture located on the south of Yunnan.

The origin of living plants of *C. globata* used for breeding experiments was not reported (Proctor 1971, 1975), but according to the context and the labels of the specimens studied, Israel might have been the locality used (see list of localities above). A strain of *C. globata* identified by Y. Lipkin was isolated from the Mamilla Pool in Jerusalem (reported with erroneous spelling as *C. globate*; Milner & Avigad 1972). Unfortunately, latter records were not consistent with herbarium specimens used in TELA and could not be verified. Although this water body still exists, it is currently inaccessible. If this population could be found it would likely be useful for future studies.

C. globata was also found in Lake Balkhash in southern Kazakhstan and in the delta of Ili River, before and at the initial stages of its damming, and also in water bodies that had disappeared within the flooding area of the Qapshaghay Bogeni Reservoir, also known as the Kapchagay Reservoir (reported with erroneous spelling as *C. globosa* Migula in several cases; Kostin & Shoyakubov 1972, 1973a,b, 1974a,b; Shoyakubov 1973, 1979). Subsequently, *C. globata* has not been listed among the species found there (Kostin 1987). Therefore, these findings require confirmation, but the presence of *C. globata* in south of central Asia remains possible.

All records, except for the first diagnosis and holotype re–examination refer to species name only and were not accompanied with locality and specimens' description and illustrations. The description and figures in "Flora Algarum Sinicarum Aquae–Dulcis" (Han & Li 1994: 235, 236, fig. 182) repeated those in "A revision of the Characeae" (Wood & Imahori 1964: Icone 87; 1965: 213).

Our study confirmed the presence of this species in western Asia (for details see Supplement S2), the eastern Mediterranean (Israel), and extended its distribution range to Europe (Russia, Krasnodar Oblast) and the Sahara–Arabian desert region (Egypt, Sinai). The new records reflect the rather poor knowledge on charophytes diversity in southeastern Europe and the Sinai Peninsula and could not be assessed as invasion cases.

C. globata has been referred to as a temperate species (Deng & Zhang 1996). All data available on its distribution are summarized in Figure 4. Notably, a recent intensive study on Iranian charophytes did not reveal this distinct species (Ahmadi et al. 2012). It might be concluded that *C. globata* has a disjunctive distribution with two main parts in arid and semi–arid regions of Central Asia and the Eastern Mediterranean.

Ecology

C. globata has been found in pools and lakes (Kostin

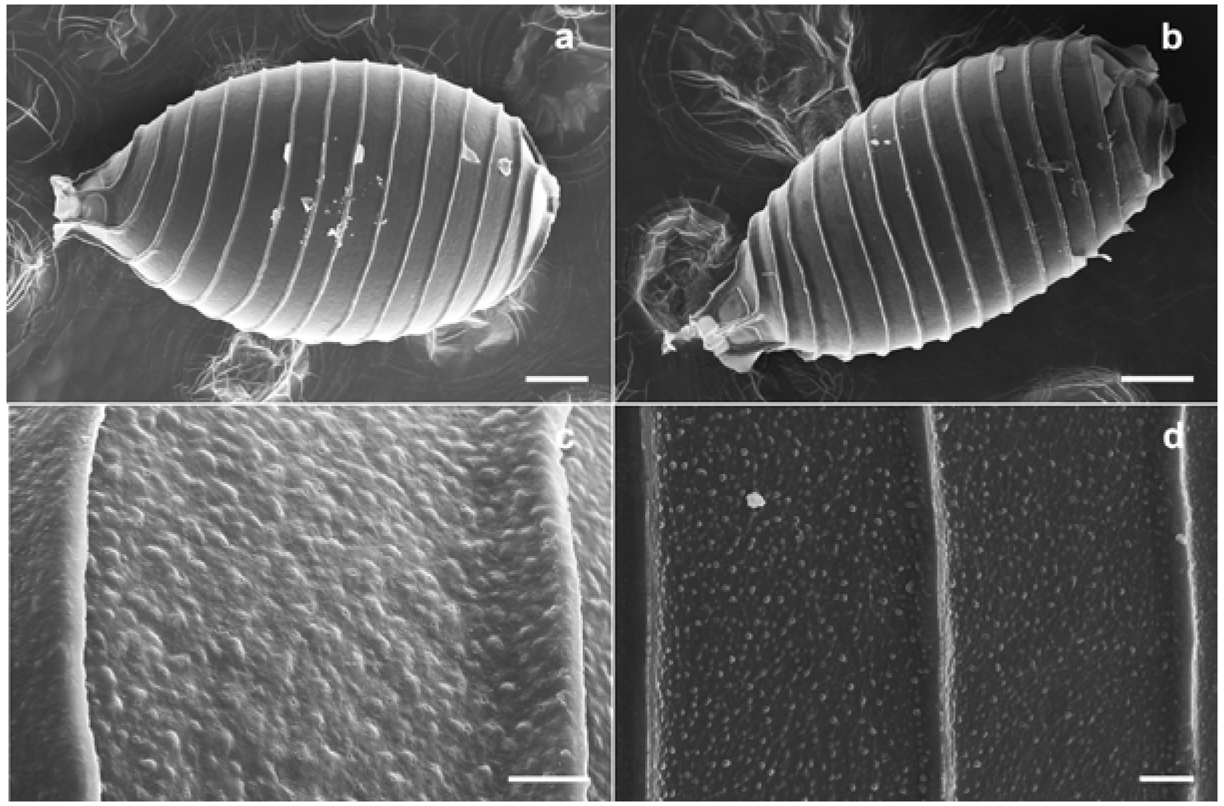

Fig. 2. The oospores of *C. globata*, SEM. Scale bar 100 µm, exept (c, d) 10 µm.

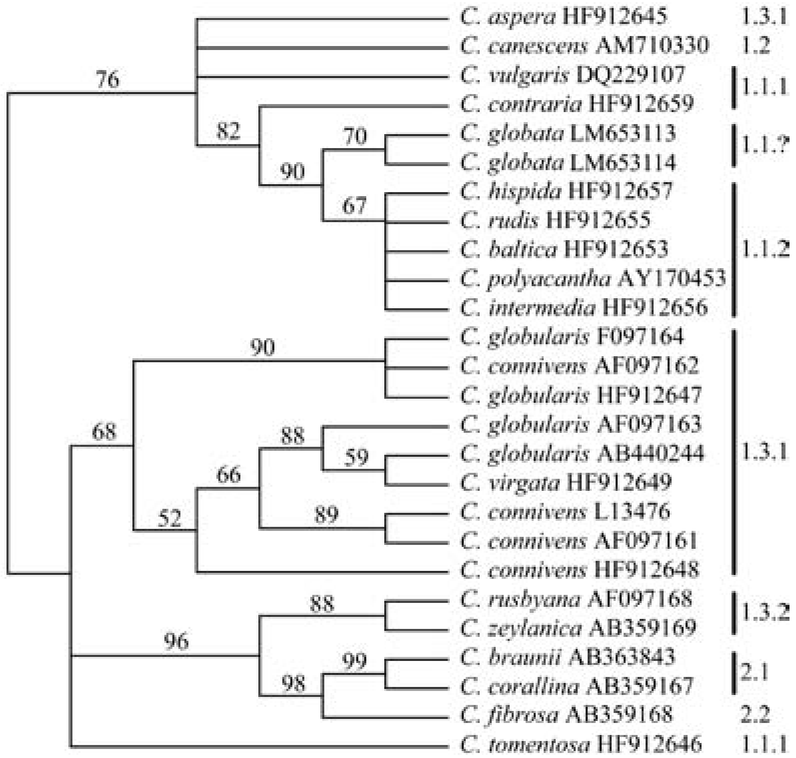

Fig. 3. MP phylogenetic tree based on 1072 aligned characters (26 MP–parsimony–informative) showing affinity of *C. globata*. Numbers above branches are bootstrap percentages [(1) subgenera *Chara* R.D. Wood: (1.1) section *Chara* R.D. Wood, (1.1.1) subsection *Chara* R.D. Wood, (1.1.2) subsection *Hartmania* R.D. Wood, (1.2) section *Desvauxia* R.D. Wood, (1.3) section *Grovesia* R.D. Wood, (1.3.1) subsection *Grovesia* R.D. Wood, (1.3.2) subsection *Willdenowia* R.D. Wood; (2) subgenera *Charopsis* R.D. Wood: (2.1) section *Charopsis* R.D. Wood, (2.2) section *Agardhia* R.D. Wood].

& Shoyakubov 1973a; 1974a,b; Ling et al. 2000), including deep pools on groundwater outputs and oxbow lake (this study), uncovered rainwater reservoirs (Milner & Avigad 1972; this study), and an inundated quarry (this study). These water bodies are situated mainly in arid and semiarid regions (i.e., in desert, steppe, and Mediterranean regions).

The environmental requirements of *C. globata* requires further study and description. According to published records, it has been found at a depth up to 2 m within a salinity range of 1.481–2.330 g.dm^{-3} (Kostin & Shoyakubov 1973a, 1974a,b). The species was successfully cultivated in an aqueous solution of water extract of soil containing sodium carbonate (20 ppm) and calcium carbonate (50 ppm). During cultivation, the pH of the media increased from 7.5 to 8.5; at a higher level (9 and more) the growth of *C. globata* was poor (Milner & Avigad 1972). Unfortunately, these records are not verifiable due to the absence of description, illustrations, and existence of voucher specimens. We can therefore conclude that *C. globata* could be a fresh–brackish moderately alkaliphilic species which prefers to grow in water containing predominately sodium sulfate, calcium, and magnesium bicarbonate. Therefore, we can preliminarily classify the environment of *C. globata* as arid–semiarid, with high insolation and middle–to–high range of water temperature all year around, with middle–to–high class of salinity according to Hustedt (1957), low or moderate alkalinity with pH of about 7.1–8.0 and low to moderately polluted nitrate concentrations (i.e. water quality class III–IV (Barinova 2011).

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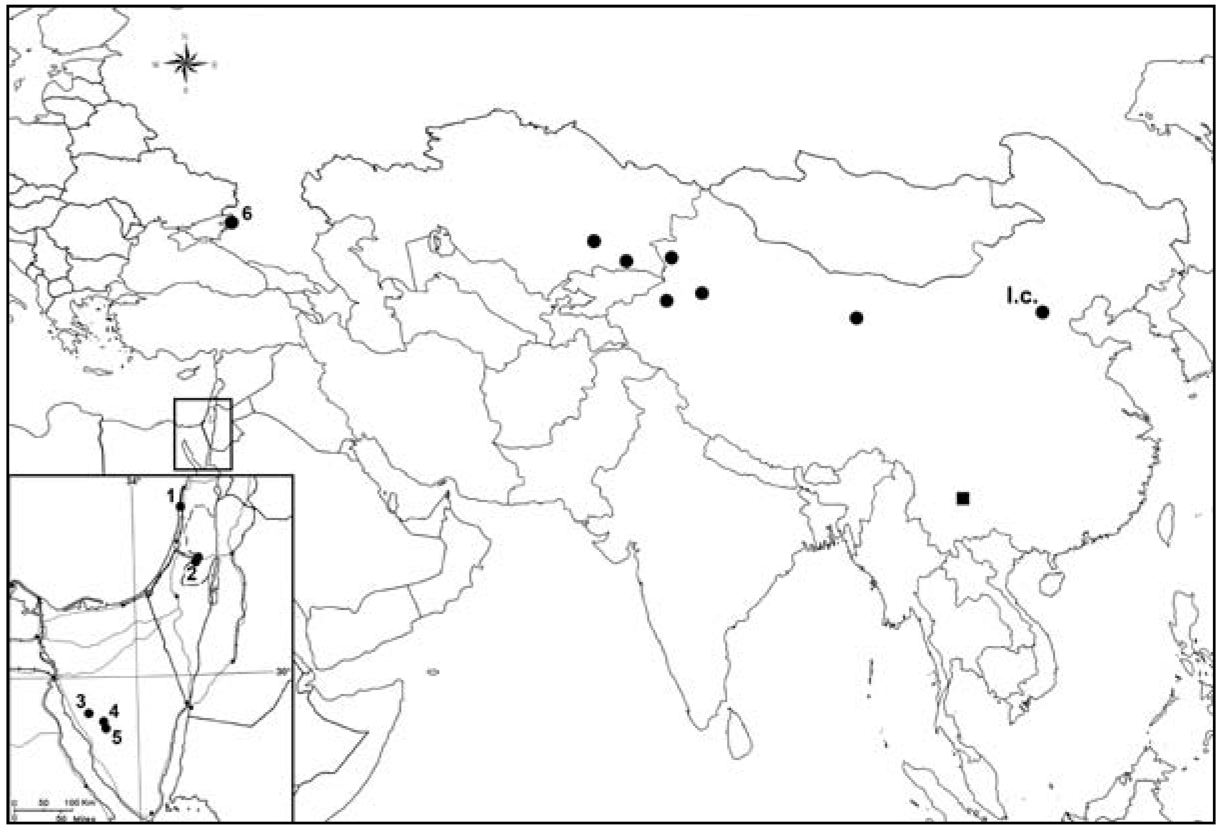

Fig. 4. Distribution of *C. globata*: (l.c.) locus classicus; (1–6) localities of specimens studied (see Supplement S2), (round points without number) localities reported, (quadrate) exact locality unknown.

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Supplementary material

the following supplementary material is available for this article:

S1. Complete morphological description of specimens studied.

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

This material is available as part of the online article (http://fottea.czechphycology.cz/contents)

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