New Record of *Chara hispida* (L.) Hartm. (Streptophyta: Charophyceae, Charales) from the Işıklı Lake (Turkey) and Critical Checklist of Turkish Charophytes

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Abstract *Chara hispida* (L.) Hartm. has been found in the Işıklı Lake (Western Anatolia). The specimens are described; distribution and ecology of *C. hispida* are discussed. The bio-indication shows that the Işıklı Lake is mainly an oligotrophic state with a trend to a mesotrophic state, and fresh low-alkaline water with low to middle organic pollution. A check-list of charophytes was compiled. There are sixteen species of charophytes known from Turkey, including 13 species of *Chara* and 3 species of *Nitella*.

Keywords *Chara hispida*, ecology, distribution, Işıklı Lake, Turkey

from drainage channels and lakes ([1-3]; this study). The exact localities of species usually were not mentioned in published data, but at least three were known from Western and Southern Anatolia and Marmara regions, respectively, in drainage channels of Kaş Ova Gelemiş in Muğla Province [4], in Salda Lake in Burdur Province [1,3] and in the vicinity of Istanbul [5]. Detailed descriptions of this species were missing, as well as its ecology and habitats. Attention was given only to a few biological activities of this species [1,3,6].

Freshwater algae are widely used as bio-indicators of aquatic environment [7], but this system is just in initial step in Turkey [8,9]. Recently, a bio-indication approach in the Eastern Mediterranean has been developed in Israel [10]. We implemented a summarized account on species autecology [11,12] for the algal community from the Işıklı Lake to characterize the environment in which *C. hispida* was found.

The aim of the present study is to describe a new record of *C. hispida*'s locality for Turkey, to characterize *C. hispida*'s environment using bioindication methods, and to compile an annotated species check-list of Turkish charophytes.

1. Introduction

Chara hispida (L.) Hartm. is widespread and relatively common in Europe. The species concepts of *C. hispida* in different regions are not identical. This mainly European species was found in the European and Asian parts of Turkey ([1-5]; this study). The Turkish localities are marginal in terms of distribution range and the frequency of occurrence. Therefore, the morphology and ecology of *C. hispida* from these border habitats deserve special attention to reveal the ranges of morphological and ecological variability and to create verifiable and reliable records of the species.

The charophyte flora of Turkey is poorly described and documented despite the comparatively large bibliography (see below). As in other regions of the Near East, the accurate and detailed documentation of species' findings in Turkey is really needed to avoid confusions and ambiguities of several species records. The lack of information is accentuated by the absence of voucher specimens or published records of its existence. To date, descriptions and illustrations of *C. hispida* specimens from the Near East exclaves are absent. In Turkey *C. hispida* had been collected

2. Materials and Methods

2.1. Description of the Study Site

Lake Işıklı (38°14' N; 29°55' E) is located in the basin of the Büyük Menderes River in the western part of Turkey (Figures 1, 2). It is an alluvium embankment in a freshwater lake formed by the Kufi stream close the riverbed of the Büyük Menderes River, which is home to numerous historic sites [13].

The river flows across the lake to the Aegean Sea and is a part of Western Anatolia in the Freshwater Ecoregions of the World (FEOW) [14]. The climate of this region is mild, having a transitional state between Mediterranean and cool,

temperate climates. The annual average air temperature is 15.8 °C (min. -11.6; max. 41.2 °C) [15]. Altitude of the Lake Işıklı is 850 m. The lake has a surface area that varies from 35 to 66.5 km² with a maximum of 73 km²; the maximum depth of the lake is 7.0 m and the mean depth is 3.77 m. The average depth of Lake Işıklı seasonally fluctuated between 1.60 and 3.15 m [16]. Lake water transparency and biomass of macrophytes also fluctuated between dry and wet seasons. As a result of the lake level's seasonal fluctuation, coastal areas are exempt from the lake water during the dry season, and the lake's shore is covered with a mass of dried plants and *Chara*. The most common macrophytes in the lake area were identified as *Chara* spp., *Ceratophyllum demersum*, *Potamogeton lucens*, *Myriophyllum spicatum*, *Polygonum amphibium*, and *Potamogeton pectinatus*. The lake water is used for the purpose of irrigation and wildlife [15].

Figure 1. Map of study site with sampling point as black circle

Chemical data for species ecology come from [16,17].

2.2. Sampling and Cleaning

Charophyte samples were taken by Dr. Erdoğan in the northeast part of the lake by scooping, fixed in formaldehyde 4% solution, and transported to the Dumlupınar University in an ice box. Part of the samples were sent to the Institute of Evolution, University of Haifa and studied under a Nikon light microscope and Leica stereomicroscope with digital cameras under magnification 400-1000. Part of charophyte samples was sent to the Central Siberian Botanical Garden and studied under a stereomicroscope Carl Zeiss Stereo Discovery V12 and micrographs were taken with the digital camera AxioCam MRS-S and Axiovision 4.8 software.

The diatom samples were collected by Dr. Erdoğan by using plankton net (25 µm mesh) from 0-70 cm depth of the lake. Periphyton from charophytes and planktonic samples were boiled in H₂O₂ and HCl to remove calcium carbonate and organic matter. After washing several times with distilled water, the material was air-dried on cover glasses and mounted in Naphrax®. The diatoms were observed with a Nikon Eclipse Ci and light Microscopes (LM) in Dumlupınar University Research Center (ILTEM).

3. Results

3.1. Description of the *Chara hispida* Specimens

Taxonomy and nomenclature of *Chara hispida* (L.) Hartman 1820 is given according to [18].

Figure 2. The Işıklı Lake study site, Turkey, on the map of Freshwater Ecoregions of the World (according to [14]). (1 – Dniester-Lower Danube, 2 – Thrace, 3 – Western Anatolia, 4 – Northern Anatolia, 5 – Central Anatolia, 6 – Southern Anatolia, 7 – Western Transcaucasia, 8 – Upper Tigris & Euphrates, 9 – Orontes, 10 – Kura-Southern Caspian).

Figure 3. *Chara hispida* habitat and plant overview. 1 – stands, 2, 3 – upper parts of plants, 4 – dry beds with *Chara hispida* as a result of water level decline. Scale bar 2 cm

Figure 4. *Chara hispida* morphology: 1 – axis and base of whorl, 2 – apex, 3 – whorl of branchlets, 4 – axial cortex, 5 – stipulodes, 6, 7 – gamentangia

Synonyms: *Chara major* Vaillant 1719; *Chara hispida* var. *maior* (L.) Hartman f. *maior* in R.D.Wood 1962, *Chara hispida* var. *maior* (L.) Hartman f. *maior* in R.D.Wood et Imahori 1965.

The plants are moderately incrustated, above 35 cm in height (only upper parts were available for study), scarcely branched (Figures 3, 4), with robust axes 0.7-1.5 mm in diameter. Axes cortex diplostichous obviously or slightly aulacanthous, on older internodes usually isostichous; spine-cells are acute usually in clusters of 3, one or rarely two of them very short papillose, others are from 0.25 to 3

times longer of axis diameter, dense on younger internodes and solitary short conical on older internodes. The stipulodes are in 2 tiers, 2 sets per branchlet, well developed, acute, similar in shape with cortex spine-cells. The branchlets are 2.7-5.6 cm in length, in 9-10 in a whorl, in 1.6-2-3-(4) times shorter of internodes, consist of 5-7 corticate segments and moderately long two-celled ecorticate end segment, and end cell is acute. The bract cells are verticillate, the anteriors elongate, and posteriors significantly shorter. The gametangia are solitary, conjoined, unripe, antheridia up to 460 mkm in diameter.

Table 1. Diversity of algae in the charophyte community of the Isikli Lake in September 2013 with abundance scores and species autecology

	Taxa	Scor	Sub	T	Oxy	Hal	pH	D	Sap	s	Aut-Het	Tro
1	<i>Chara hispida</i> (L.) Hartman	6	B	-	st	i	-	-	x-b	0.9	-	-
2	<i>Brachysira vitrea</i> (Grunow) Ross in Hartley	1	B	-	-	i	ind	-	o-x	0.7	-	-
3	<i>Cocconeis placentula</i> Ehrenberg var. <i>placentula</i>	1	P-B	temp	st-str	i	alf	es	o-b	1.4	ate	e
4	<i>Cocconeis placentula</i> var. <i>euglypta</i> (Ehrenberg) Grunow	1	P-B	temp	st-str	i	alf	sx	b	-	ate	e
5	<i>Cocconeis placentula</i> var. <i>lineata</i> (Ehrenberg) van Heurck	1	P-B	-	st-str	i	alf	sx	x-o	0.4	ate	e
6	<i>Cymbella cymbiformis</i> Agardh	1	B	temp	str	i	neu	sx	o	-	ats	o-m
7	<i>Cymbella hustedtii</i> Krasske	1	B	-	str	i	alf	-	o	-	ats	o-m
8	<i>Cymbella lange-bertalotii</i> Krammer	2	B	-	st-str	i	alf	sx	o-b	1.5	ats	e
9	<i>Cymbella vulgata</i> Krammer	1	-	-	-	-	-	-	-	-	-	-
10	<i>Denticula kützingii</i> Grunow	2	B	-	str	i	alb	-	o-b	1.5	ats	m
11	<i>Encyonema caespitosum</i> Kützing	1	B	-	-	-	-	sx	b-a	-	-	-
12	<i>Encyonopsis eifelana</i> Krammer	1	-	-	-	-	-	-	-	-	-	-
13	<i>Encyonopsis microcephala</i> (Grunow) Krammer	2	B	-	str	i	alf	es	b	-	ats	me
14	<i>Encyonopsis minuta</i> Krammer et Reichardt	6	B	-	-	oh	-	-	-	-	-	-
15	<i>Encyonopsis moseri</i> Krammer et Lange-Bertalot	1	-	-	-	-	-	-	-	-	-	-
16	<i>Encyonopsis</i> sp.	1	-	-	-	-	-	-	-	-	-	-
17	<i>Encyonopsis subminuta</i> Krammer et Reichardt	1	B	-	st-str	i	ind	sx	x-o	1.3	ate	o-e
18	<i>Epithemia adnata</i> (Kützing) Brébisson	1	B	temp	st	i	alb	sx	b-a	2.5	ats	me
19	<i>Epithemia smithii</i> Carruthers	6	B	-	-	-	-	sx	-	-	-	-
20	<i>Eunotia arcus</i> Ehrenberg	2	B	-	-	i	acf	-	x-b	0.8	ats	o-m
21	<i>Gomphonema</i> sp.	1	-	-	-	-	-	-	-	-	-	-
22	<i>Mastogloia dansei</i> (Thwaites) Thwaites ex W.Smith	1	B	-	-	mh	alf	-	-	-	-	-
23	<i>Mastogloia elliptica</i> (Agardh) Cleve	4	B	-	-	mh	alf	-	-	-	-	-
24	<i>Mastogloia lacustris</i> (Grunow) Grunow	1	B	-	str	hl	alf	-	o	1	ats	e
25	<i>Navicula cryptotenelloides</i> Lange-Bertalot	1	B	-	-	oh	alf	-	b-a	-	-	-

Note: Substrate (Sub) – substrate preferences (P-B – plankto-benthic, B – benthic); Temperature (T) – temperature preferences (temp – temperate); Oxygenation (Oxy) – streaming and oxygenation (st – standing water, str – streaming water, st-str – low streaming water); Salinity (Hal) – halobity degree according to [42] (i – oligohalobes-indifferent, mh – mesohalobes, hl – halophiles; oh – undifferent oligihalobes); Acidity (pH) – pH degree according to [43] (alb-alkalibiontes; alf – alkaliphiles, ind – indifferents; acf – acidophiles; neu – neutrophiles as a part of indifferents); Saprobity DALPO (D) – degree of saprobity according to [44] (sx – saproxenes, es – euryasaprobies); Autotrophy-Heterotrophy (Het) – nitrogen uptake metabolism [11] (ats – nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; ate – nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen); Trophic (Tro) – trophic state [11] (o-m – oligo-mesotrophic; m – mesotrophic; me – meso-eutrophic; e – eutrophic; o-e – oligo- to eutrophic (hyper-eutrophic)); Saprobity S (Sap) – degree of saprobity [45] (x-o – xeno-oligosaprobies, o-x – oligo-xenosaprobies, x-b – xeno-betamesosaprobies, o – oligosaprobies, o-b – oligo-betamesosaprobies, b – betamesosaprobies, b-a – beta-alphamesosaprobies); s – species-specific saprobity index [12].

3.2. Habitat Description

The Işıklı Lake, on depth 0-70 cm, with average values of 15.35 kg·m⁻² (wet) and 2.0 kg·m⁻² (dry) in environmental condition as: pH 8.42-7.85, Conductivity 329-448 μS/cm, Temperature 5.6-28.1°C with average 15.6 °C, Dissolved Oxygen 6.57-10.96 mg L⁻¹, Chloride 19.65-13.48 mg L⁻¹, Organic matter 26.70-19.93 mg L⁻¹, NTU 2.53-8.07, Bicarbonate 158.60-252.26 mg L⁻¹, Carbonate 3.16-17.85 mg L⁻¹, Hardness 21.01-30.76 °F, Calcium 43-08-82.79 mg L⁻¹, Magnesium 25.92-29.78 mg L⁻¹, Nitrate 0.91-1.01 mg L⁻¹, Sulfate 36.94-67.13 mg L⁻¹, Phosphate 0.06-0.08 mg L⁻¹, Ammonia 0.41-1.18 mg L⁻¹, Hydroxyl 0.00 mg L⁻¹, Salinity 0.00 %, Carbon dioxide 0.53-1.08 mg L⁻¹, together with aquatic plants *Ceratophyllum demersum*, *Potamogeton lucens*, *Myriophyllum spicatum*, *Polygonum amphibium*, and

Potamogeton pectinatus [16]. The habit of studied specimens likely reflects comparatively low-light intensity in *C. hispida*'s habitat [19].

3.3. Distribution of *C. Hispida*

Species is widespread and relatively common in Europe, in Northern Europe its distribution connected to limestone areas [20]. *Chara hispida* had been found in nearly all states in Europe, i.e. in Serbia, Croatia, Macedonia, Slovenia, Bosnia and Herzegovina, Bulgaria, Greece [21], Romania [22], Czech Republic [23], Slovakia [24], Austria [25], Latvia [26], Lithuania [27], Belgium [28], Netherlands (as *C. major* (Hartm.) Hy [29], Switzerland [30], Germany [31], Poland [32], Russia [33], Kazakhstan [34], Denmark, Norway, Sweden [20], Portugal, Spain, France, Hungary,

Italy, Ukraine [35], Great Britain, Ireland [36]. In Asia *C. hispida* had been reported from Egypt [37], Oman [38], Jordan [37], Turkey [5,35], Azerbaijan [33], Kazakhstan [39] and Russia [5]. In Africa it is known from Atlas Mountains in Morocco [40] Algeria and Tunis [41]. So it may be concluded that *C. hispida* have mainly European areal with few exclaves in Northern Africa, Near East, Caucasus, Central Asia and Eastern Siberia, but revision of several records outside of main part of its areal is needed.

3.4. Bio-indication of the *C. hispida* Environment

We use bio-indication methods on the basis of algal and invertebrate species, which inhabit lake water and substrates, for the purpose of characterizing the water quality of the lake in which *C. hispida* grows.

Altogether, twenty-five algal species were occupied the *C. hispida* plants. We found diatom species only (Table 1). Most abundant in the periphytic community were *Encyonopsis minuta* Krammer et Reichardt, and *Epithemia smithii* Carruthers. Periphytonic algae characterize the environment in which *C. hispida* inhabits, such as temperate, low alkaline, middle oxygenated fresh waters with low to middle organic pollution. As can be seen in Table 1, algal species survived mostly as benthic forms, but plankto-benthic life forms was also presented. Species-specific index of saprobity fluctuated from 0.7 to 2.5. We calculated the Index of saprobity S for the algal community according to [46] and found it as 1.13, which reflects Water Quality Class II. In this way, we analyzed species nutrition preferences (Table 1), and found algal species used only photosynthesis for biomass production; however, mixotrophic species were absent. Bio-indication can help us to assess a trophic state of the lake (Table 1) and shows that Işıklı is an oligotrophic lake. Lacoul & Freedman [47] characterized *C. hispida* as an oligotraphent species, which have species optimum in an oligotrophic lake environment.

Therefore, bio-indication shows that Işıklı Lake mainly has an oligotrophic state with a trend to the mesotrophic state, and fresh low-alkaline water with low-to-middle organic pollution.

4. Discussion

4.1. Ecology and Habitats

Chara hispida is one of the largest species of charophytes; usually its thallus height is between 0.3-2 m [35], and occasionally individual plants may be 3.5 m in length [48]. It often forms dense perennial beds [36] with depths up to 15 m, mainly between 0.5-2 m [35] or 2-7 m [49]; annual forms are known from shallow water [20,50]; sometimes its pure stands are formed in groundwater output [35]. *Chara hispida*

beds may form a significant part of clear-water calcareous lake vegetation; this is one of the key species in "Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp." according to the European Union Habitat Directive (NATURA 2000 Code: 3140). Its abundance and vitality may be used as an indicator of favorable conditions in these ecosystems [51]. *Chara hispida* beds could be acting as nitrogen sinks in several lakes [52]. Its stands create a specific habitat for plankton, particularly via space structuring and refuge forming for zooplankton [53] and in the formation of allelochemicals, probably impacting phytoplankton [54].

Chara hispida is eurythermal, alkalifilic [49], oligotraphentic [47,55], and indifferent to light intensity species [49,56]; it prefers calcareous-oligosaprobic waters [35].

This species grow in freshwater calcareous alkaline pools and lakes [20,36] as well as in small water bodies [50], peat bogs and water holes in calcareous fens [35], including habitats, which dry out for short periods [36]. Occasionally, *C. hispida* occurs in brackish waters [50,57] therefore, it may be characterized as indifferent to salinity [50]. It may be concluded that *Chara hispida* may grow in a comparatively wide range of water-body types. It frequently shares the same habitats with other species of *Chara* and *Nitellopsis obtusa* (Desv. in Loisel.) J. Groves. Moreover, it seems extremely problematic to achieve clear habitat specificity differentiation from other species of *Chara* as well as to attempt to explain the extant distribution of *C. hispida* through preferred habitat types.

Chara hispida as another large species of charophytes that can't successfully persist in shallow waters, which is likely due to the high susceptibility to mechanical damage by waves and ice cover; its distribution is restricted to deeper water [58]. Therefore, it may completely disappear from a water body after water transparency decreases as a result of eutrophication [58] and sometimes can't reappear and regenerate its own community after restoring previous low-trophic levels of the water ecosystem [59]. Its successful recolonization of clear-water lakes, shifting from turbid plankton-dominated state, is known from the Netherlands [60] and Sweden (Blindow, cit. [51]).

4.2. Check-list of Turkish Charophytes

We shared published information about charophyte species distribution in the territory of Turkey for the purpose of finding localities of the studied species *C. hispida* in the list of Turkish Charales. Unfortunately, less than half of the charophyte species reported from Turkey was included in the last check-list of algae [62]. Therefore, we compiled a preliminary check-list of charophytes for Turkey, excluding records of *Chara* sp. and *Nitella* sp. The 13 species of *Chara* and 3 species of *Nitella* had been reported from Turkey, which is similar to Israel species richness with 15 charophyte

species and one variety [63,64]¹, Iran [65-69] with 19 species and two varieties, and Georgia with 14 species [70], and contrary to dry climatic habitats in Arabian Peninsula with 11 species and two varieties in Saudi Arabia [71-73], and eight charophyte species and one variety in Oman [67,74].

Below we presented an annotated check-list with the references included in our findings of *C. hispida*. The revision of several records is needed.

1. *Chara aspera* Willd. (as *C. globularis* Thuill. var. *aspera* R.D. Wood, [2,4].
2. *C. canescens* Desv. et Loisel. in Loisel. [62,75,76].
3. *C. globularis* Thuill [2,4,62,76], as *C. fragilis* f. *hedwigii* (C.Ag. in Bruz.) Kütz., [5]; as *C. fragilis* Desv. [77].
4. *C. gymnophylla* A.Br. [78], as *C. foetida* subsp. *gymnophylla* β *fontanesiana* A. Br., [5]; as *C. vulgaris* var. *gymnophylla* (A.Br.) Nym. [62].
5. *C. hispida* L. [1-6,35,50,78,79], this study.
6. *C. inconnexa* T.F. Allen (as *C. vulgaris* var. *inconnexa* (T.F. Allen) R.D. Wood, [2,4,62].
7. *C. intermedia* A.Br. [5,36,78].
8. *C. kieneri* Daily (as *C. vulgaris* f. *kieneri* (Daily) Norton ex R.D. Wood, [62].
9. *C. tenuispina* A.Br. [35].
10. *C. tomentosa* L. [2,4,35,79], as *C. ceratophylla* Wallr. [5,78].
11. *C. virgata* Kütz. (as *C. globularis* var. *virgata* (Kütz.) R.D. Wood, [2,4].
12. *C. vulgaris* L. [2,4,62,76,80-83], as *C. foetida* A.Br., [5]; as *C. foetida* (*subhispida*) [77].
13. *C. zeylanica* Klein ex Willd. [2,5].
14. *Nitella gracilis* (Sm.) C. Agardh [2]; also *N. cf. gracilis* had been reported [5].
15. *N. mucronata* (A.Br.) Miq. in H.C. Hall [35,78,84], as *N. mucronata* var. *virgata* (Wallm.) A.Br. [5].
16. *N. opaca* C. Agardh [81,85], as *N. opaca* f. *heteromorpha* Mig. [80].

5. Conclusion

Charophyte species *C. hispida*'s beds represent one of the less studied habitats in the Işıklı Lake. A detailed study of morphology, ecology, and distribution of *C. hispida* confirm species ecology as inhabiting oligotrophic freshwater, low-alkaline lakes with low-to-middle organic pollution. This species can form a large biomass as a result of growth in a stable environment. Periodically exposing the lake bedrocks as a result of the lake level's seasonal fluctuation is obviously the cause of dead *C. hispida* thalli. But the life cycle of *Chara* with oospores' formation helps in renewing the algal growth during the wet season with restoration of the large biomass, likely with average values 15.35 kg m⁻² (wet)

and 2.0 kg m⁻² (dry) [as *Chara* spp., 15]. Therefore, we can conclude that the studied lake has optimal environment for growing of *C. hispida* in Turkey. In this case we recommended the Işıklı Lake to be included in the Turkish net of protected areas with regular monitoring of chemical and biological variables, and watershed protection.

Acknowledgements

We give thanks to Dr Omer Erdoğan from the Süleyman Demirel University, Isparta, Turkey, for his help on collecting samples in the lake. This work has been partly funded by Israel Ministry of Absorption.

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¹ A comprehensive bibliography for Israel, Iran, Georgia and Saudi Arabia charophytes is impossible to report within the scope of this article, so here and below are the cited references of those that include all known species from the territory.

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