

# New national species records of charophytes (Characeae) for Montenegro, Croatia, and Cyprus: update of rare species distribution in Southeast Europe

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**Key words:** *Chara*, *Sphaerochara*, *Tolypella*, floristic novelties, protection status

**Ključne besede:** *Chara*, *Sphaerochara*, *Tolypella*, floristične novosti, varstveni status

## Abstract

Field studies in 2022–2024 and the checking of collection of the Natural History Museum of Montenegro revealed seven new national species records for Montenegro: *Chara corfuensis* Groves ex Fil., *C. galioides* DC., *C. squamosa* Desf., *Sphaerochara intricata* (Trentep. ex Roth) Feist-Castel & N. Grambast, *Tolypella hispanica* Nordst. ex Allen, *T. nidifica* (O. F. Müll.) A. Braun and “Mediterranean *Chara*”. The checking of the specimens stored in ZE Botanischer Garten und Botanisches Museum, Freie Universität Berlin, Komarov Botanical Institute of RAS, and Naturhistorisches Museum Wien revealed old specimens of *C. galioides*, a new species for Croatia, and new sites of *T. hispanica* for Greece. The field research in Cyprus resulted in a new national species record of *C. squamosa*. New data enriched knowledge about the distribution of charophytes in Central and Eastern Mediterranean and Southeast Europe and allowed tentative suggestions of important charophyte areas in Montenegro. The tentative evaluation of species protection statuses was implemented here. The updated species list of Montenegrin charophytes counted 37 species making Montenegro one of the charophyte species-rich countries in Europe.

## Izvleček

S terenskimi raziskavami med letoma 2022 in 2024 in pregledom zbirk v Prirodoslovnem muzeju Črne gore smo odkrili za Črno goro sedem novih vrst: *Chara corfuensis* Groves ex Fil., *C. galioides* DC., *C. squamosa* Desf., *Sphaerochara intricata* (Trentep. ex Roth) Feist-Castel & N. Grambast, *Tolypella hispanica* Nordst. ex Allen, *T. nidifica* (O. F. Müll.) A. Braun in “Sredozemska *Chara*”. S pregledom primerkov, shranjenih v zbirkah ZE Botanischer Garten und Botanisches Museum, Freie Universität Berlin, Komarov Botanical Institute of RAS in Naturhistorisches Museum Wien, smo odkrili star primerek vrste *C. galioides*, nove vrste za Hrvaško in nove lokalitete vrste *T. hispanica* v Grčiji. S terenskimi raziskavami na Cipru pa smo odkrili novo lokaliteto vrste *C. squamosa*. Novi podatki so obogatili poznavanje razširjenosti parožic v srednjem in vzhodnem Sredozemlju ter jugovzhodni Evropi, hkrati pa izpostavili pomembna območja parožic v Črni gori. V članku smo ovrednotili začasni varstveni status vrst. Posodobljen seznam sedaj vsebuje 37 vrst in Črna gora je tako ena od držav v Evropi z največ vrstami parožic.

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## Introduction

The charophytes (Charophyta, Charophyceae, Characeae) are a widely distributed group of macroscopic algae associated with continental water bodies. Plant arrangement allows easy recognition of this group from first glance. However, species identification still can be an inspiring challenge because of insufficiently known variability of some species and still debated species concepts (Schubert et al., 2024). Besides the importance of charophytes as keystone species for some types of ecosystems, they are important species in threatened and protected habitats, e.g. “3140. Hard oligo-mesotrophic waters with benthic stoneworts *Chara* spp.”, “3170. Mediterranean temporary ponds”, and “3190. Lakes of gypsum karst” of the Habitat Directive by European Union and “C1.2a. Permanent oligotrophic to mesotrophic waterbody with Characeae”, a vulnerable habitat type according to the European Red List of Habitats (European Commission, 2017). Mediterranean temporary ponds appear to be one of the most precious habitat types in the south of Europe because they harbor rare and peculiar species (Bagella, 2023). Charophytes are one of the most threatened groups of plants in Europe (Baastrup-Spohr et al., 2013), which can be easily illustrated by the high proportions of endangered species in many national and preliminary European Red Lists (Becker, 2016; Stewart, 2024). The key areas important for protecting of vulnerable and endangered species were outlined in a few European countries only (e.g. Blaženčić et al., 1998; Stewart, 2004). Therefore, accurate and reliable datasets about species distribution in time and space are essential for monitoring and designing natural protected areas avoiding already known irreversible loss of important sites (Langangen, 2017; Troia, 2020). Despite long history of research on charophyte species distribution in Central Mediterranean and Southeast Europe (see Blaženčić et al., 1997, 1998, 2006a, b; Biberdžić et al., 2006; Bazzichelli & Abdelahad, 2009; Biberdžić & Blaženčić, 2013; Blaženčić, 2014; Troia et al., 2018; Becker, 2019) even national species lists are not complete. In the last decade, new national species records are added almost every year (Zeneli & Kashta, 2016; Troia et al., 2018; Becker, 2019; Romanov, 2019; Romanov et al., 2019; Trbojević et al., 2020, 2024; Becker et al., 2021; Koletić et al., 2021; Casanova & Becker, 2022; Biberdžić et al., 2023; Schubert et al., 2024). Montenegro was already recognized as one of the important areas of charophyte species diversity in Europe (Blaženčić & Stevanović, 2015) partly because of the high species richness in Lake Skadar (Blaženčić et al., 2018). According to the contemporary nomenclature (Schubert et al., 2024), 30 species are reported for Montenegro.

This research aimed to update the national species lists adding seven novel species records for Montenegro, one new species for Croatia and Cyprus respectively, enriching knowledge of charophyte species distribution in Central and Eastern Mediterranean and Southeast Europe, and assessing their national protection statuses.

## Material and Methods

During 2022–2024, a survey of different aquatic habitats of Montenegro, mostly on the Adriatic coast from the westernmost to easternmost extremities of the country, was carried out, as well as an analysis of samples from the collection of the Natural History Museum of Montenegro (NHMM). Most areas of the Adriatic coast and some inland sites looking suitable for charophytes at satellite images were checked several times in different seasons. It allowed tentative estimation of the duration of the existence of most temporal water bodies and the stability of charophyte populations. The specimens were collected by hand and grapnel from water bodies by the authors. The rough assessment of the water salinity was based on the species and abundance of angiosperms and macroscopic algae associated with charophytes in Montenegrin sites. Electric conductivity and pH were measured by Thermo Scientific™ Elite PCTS (Thermo Fisher Scientific Inc., Waltham, MA USA) after 2 days of sampling in Cyprus, where some plants were collected by Martin Dobrota. They were cultivated at room temperature for 10 weeks to trace stem cortex traits. The specimens were pressed or stored in 70% ethanol. They were deposited in the herbarium of the NHMM. The collections of Naturhistorisches Museum Wien (W), ZE Botanischer Garten und Botanisches Museum, Freie Universität Berlin (B), Naturalis Biodiversity Center (L) and the Komarov Botanical Institute of the Russian Academy of Sciences (LE) were checked by the first author in search of specimens from Central and Eastern Mediterranean, and Southeast Europe. The morphological features of the specimens were examined using a stereo microscope Olympus SZX12, and microscopes Olympus BX51 and BH2 (Olympus Corporation, Shinjuku, Tokyo, Japan). Species distribution in Southeast Europe was illustrated with maps prepared with SimpleMappr (Shorthouse, 2010). Nomenclature follows the monograph “Charophytes of Europe” (Schubert et al., 2024). The tentative assessment of protection statuses was implemented according to IUCN criteria (IUCN, 2012) and the suggestions focused at charophytes by Stewart (2024). However, further researches in the region are needed for a stable assessment of the IUCN categories.

## Results and discussion

During our field survey, more than 220 sites (18 species and 5 genera) were found mostly on the Montenegrin Adriatic coast. Seven species listed below are new national records for Montenegro. One species is a new national record for Croatia, and one species is a floristic novelty for Cyprus. Some Greek sites for two species missed in recent maps of species distribution in Europe (Lambert & Schubert, 2024a; Troia et al., 2024) were traced in studied herbarium collections. Results are here reported by taxon, in alphabetical order.

***Chara corfuensis*** Groves ex Fil. (Figure 1a), Montenegro, Ulcinj Municipality, northeast vicinities of Velika plaža (Long Beach): 1. bare Đurišića, elongated sand pit, 41.88200 N 19.33840 E, 30.08.2023; 2. deep sand pit, 41.88600 N 19.33020 E, 30.08.2023; 3–5. small interdunal ponds in a large sand pit with a lot of silty sediments, 41.88780 N 19.32180 E, 41.88790 N 19.32170 E, 41.88460 N 19.33140 E, 22.09.2023.

This species was spotted in stands forming at the deepest parts of plain freshwater interdunal ponds and inundated sand pits with prominent seasonal changes in water levels increasing during winter and severely dropping during summer to ca. 50 cm and less depth. *Chara corfuensis* was found in freshwater water bodies in Montenegro as in Croatia, and the North Black Sea region (Blaženčić, 2006; Romanov et al., unpubl. data; this research) in contrast to mostly brackish lagoons and lakes in Albania and Greece (Christia et al., 2024). The preference for small ponds with highly variable water levels in Montenegro can be evidence of the susceptibility of this species to interannual differences in weather and climate change. The development of Velika plaža vicinities, including urbanization and spontaneous dumping as well as sand extraction, resulting in physical disturbance, fragmentation and loss of habitats can be recognized as a threatening factor suitable for all charophyte species in this area. *Chara corfuensis* can be referred to “Endangered” (IUCN criteria B1, 2a,b, C2a, D) in Montenegro. It was recognized as “Critically Endangered” in the Red List of Balkan charophytes (Blaženčić et al., 2006b) and “Endangered (European and Globally)” in the provisional European Red List (Stewart, 2024).



**Figure 1:** The general habit of species from Montenegro: A – *Chara corfuensis* having a spiny outline because of long pointed bract-cells, spine-cells, and stipulodes, B – “Mediterranean *Chara*” having a less spiny outline, C – male plants of *Chara galioides*, D – female plants of *Chara galioides*. Arrowheads indicate female plants, subtle and less conspicuous in comparison to rough male plants bearing large orange antheridia. Photographs by R. E. Romanov.

**Slika 1:** Splošna podoba vrst v Črni gori: A – *Chara corfuensis* ima trnast obris zaradi dolgih špičastih braktejnih celic, trnskih celic in paprilističev, B – “Sredozemska *Chara*” ima manj trnast habitus, C – moške rastline vrste *Chara galioides*, D – ženske rastline vrste *Chara galioides*. Puščice kažejo manj opazne ženske rastline, saj imajo moške rastline velike oranžne anteridije. Fotografije R. E. Romanov.

In Southeast Europe, *Chara corfuensis* is known from Albania, Croatia, and Greece from very few sites (Figure 2a; Blaženčić, 2006; Langangen 2012 and references herein; Christia et al., 2024 and references herein). It is distributed in Southern Europe, mainly in the Mediterranean, but it is known from the Atlantic coast and North

Black Sea region too (Christia et al., 2024 and references herein; Romanov et al., unpubl. data). It belongs to the subsection *Hartmania* R.D. Wood, or in other words, to the group trickiest for the delineation and identification in the Mediterranean (Blindow et al., 2024b).



**Figure 2:** Distribution of charophyte species in Southeast Europe and Cyprus based on available data according to time intervals in the monograph “Charophytes of Europe” (Schubert et al., 2024; see references in the text). A – *Chara corfuensis*, B – *Chara galioides*, C – *Chara squamosa*, D – the taxon of uncertain affiliation “Mediterranean *Chara*”, E – *Tolypella hispanica*, F – *Sphaerochara intricata*, G – *Tolypella nidi-fica*. Black dots are records from 1980 on, red dots from 1950 to 1979, and yellow dots before 1950.

**Slika 2:** Razširjenost parožic v jugovzhodni Evropi in na Cipru, prikazana na osnovi podatkov iz monografije “Charophytes of Europe” (Schubert et al., 2024; glej reference v tekstu) v časovnih intervalih. A – *Chara corfuensis*, B – *Chara galioides*, C – *Chara squamosa*, D – takson z nejasno pripadnostjo “Sredozemska *Chara*”, E – *Tolypella hispanica*, F – *Sphaerochara intricata*, G – *Tolypella nidi-fica*. Črne točke so podatki od leta 1980 naprej, rdeče točke med letoma 1950 in 1979 in rumene točke pred letom 1950.

*Chara galioides* DC. (Figure 1c, d), Montenegro, Ulcinj Municipality. Vicinities of Velika plaža, temporary ponds:

1. inundated shallow depression in community of *Juncus acutus* L. near *Tamarix* sp., ca. 41.87864 N 19.33009 E, 14.01.2023; 2. inundated shallow depression in the community of *Juncus acutus*, 41.88926 N 19.30924 E, 11.02.2023; 3. margin of the inundated dirt road, 41.88821 N 19.31089 E, 11.02.2023; 4. a puddle at dirt road, 41.87237 N 19.33935 E, 22.02.2023; 5. inundated shallow depression in the community of *Juncus acutus*, 41.87838 N 19.32989 E, 22.02.2023; 6. inundated channel, 41.87873 N 19.33600 E, 22.02.2023; 7. inundated shallow depression W of the road, 41.91603 N 19.33852 E, 23.03.2023; 8. inundated shallow depression in the community of *Juncus acutus*, 41.87928 N 19.32992 E, 17.04.2023; 9, 10. inundated dirt road, 41.87202 N 19.33910 E and 41.88003 N 19.33110 E, 25.03.2023; 11–15. vicinity of the beach of Copacabana, 41.88889 N 19.30902 E, 41.88937 N 19.30865 E, 41.89279 N 19.30923 E, 41.89309 N 19.30940 E, and 41.88889 N 19.30902 E, 17.04.2023; 16. inundated sand pit, 41.8820 N 19.3387 E, 30.08.2023. Ulcinj municipality, NE of the Nature Park “Ulcinjaska Solana” (Ulcinj Saline): 17. Knet a ispod Darze, temporary pond, 41.94066 N 19.31667 E, 02.03.2023. 18, 19. inundated field near the channel, 41.94173 N 19.32634 E and 41.94165 N 19.32614 E, 01.05.2024. Ulcinj municipality, Ulcinjska Solana: 20–35. temporary ponds in abandoned crystallization and evaporation basins: 41.92788 N 19.27491 E, 17.04.2023; 41.91960 N 19.25330 E, 30.04.2023; 41.91785 N 19.32479 E, and 41.92040 N 19.25210 E, 06.05.2023; 41.9114 N 19.3262 E, 41.9139 N 19.3286 E, 41.9142 N 19.3269 E, and 41.9145 N 19.3265 E, 23.05.2023; 41.92155 N 19.33144 E, 41.92162 N 19.33099 E, 41.92069 N 19.33163 E, 41.91922 N 19.33114 E, 41.91898 N 19.33167 E, 41.91895 N 19.33190 E, 41.91864 N 19.33130 E, and 41.91779 N 19.33040 E, 19.05.2024.

Croatia. 1. In mit süßem Wasser erfüllten \*\*\* [illegible words] Insel San Pietro di Nembi im Quarnero, [24 April 1862, H.W. Reichardt. Det. A. Braun: *Chara aspera*. 2. Istrien. In einer Süßwasserlake der Insel San Pietro di Nembi, 24 April 1862, H.W. Reichardt. Det. [A. Braun]: *Chara aspera*. 3. San Pietro di Nembi im Quarnero, [24 April 1862], H. W. Reichardt. Det. [A. Braun]: *Chara aspera*. Det. R. E. Romanov: *Chara galioides* (W, three sheets). 4. Quarnero. In einer Süßwasserlake \*\*\* [illegible words] San Pietro di Nembi, 24 April 1862, H. W. Reichardt. Det. [A. Braun]: *Chara aspera*. Det. R. E. Romanov: *Chara galioides* (B 35797).

Greece. Attica: in aquis salsuginosis stagnatibus, ad Phalerum. Legimus foem. 16 Mai et marem 19 Mai 1886. De Heldreich Herbarium Graecum Normale. 995. Det.: *Chara crinita* Wallr., Sydow Charac. p. 52, Nym. Concept. 876, No. 18. forma b. *polysperma* Al.Br. f. *longifolia* Syd. l. c., *laxius aculeata*, *valde incrustata* Sydow! in litt. 1887. Mas et foemina. Det. U. Raabe: *Chara galioides* 12.01.2000. Conf. R. E. Romanov (W 536, W 537).

This is a common species in the south-easternmost area of Montenegro, in plain small temporary brackish to nearly freshwater water bodies in less disturbed areas of Velika plaža and in some inundated crystallization and evaporation basins of Ulcinjska Solana. It occurs slightly less frequently in water bodies in the northeastern and eastern vicinities of Ulcinjska Solana. In contrast, in Montenegro, it was not found west of this area. All spotted populations are consisted of annual plants appearing during January and February and sterile during this period, but richly fertile during spring and summer. The gametangia appear not early during plant germination and the beginning of growth despite annual populations in temporary water bodies mainly disappearing at the end of spring and summer. The antheridia appear earlier than oogonia. This species can be spotted as mostly dry plants at dry bottoms for a short period after water evaporation during summer. In Croatia, it is known only from the collection of the 19<sup>th</sup> century from the small Adriatic island of Sveti Petar. *Chara fragilis* Desv. var. *brevibracteata sine auctoris* was reported from this island. It was spotted on the same visit by the same collector (Reichardt, 1863). It is unclear if it was based on the same specimens of *Chara galioides* or if it was true *Chara globularis* Thuill. considering the small size of this dry Mediterranean island and the existence of the specimen of *Chara globularis* collected from the same island on 20 May 1867 (B: 35811). The specimens by Reichardt were referred by A. Braun to *Chara aspera* Willd. with some doubts (Leonhardi, 1864). The checking of old herbarium specimens stored in W allowed adding one site for Greece (Figure 2b) missed on the last map of species distribution in Europe (Lambert & Schubert, 2024a).

The preference of the species for temporary ponds can be evidence of the susceptibility to interannual differences in weather and climate change. The urbanization of Velika plaža vicinities resulting in physical disturbance and loss of habitats can be recognized as a threatening factor. Considering the number of sites in Montenegro in comparison to few other sites in Southeast Europe the surveyed area has an international importance for the protection of this species. *Chara galioides* can be referred to “Near Threatened” (IUCN criteria B2a,b, C2a, D) in Montenegro and “Critically Endangered” in Croatia

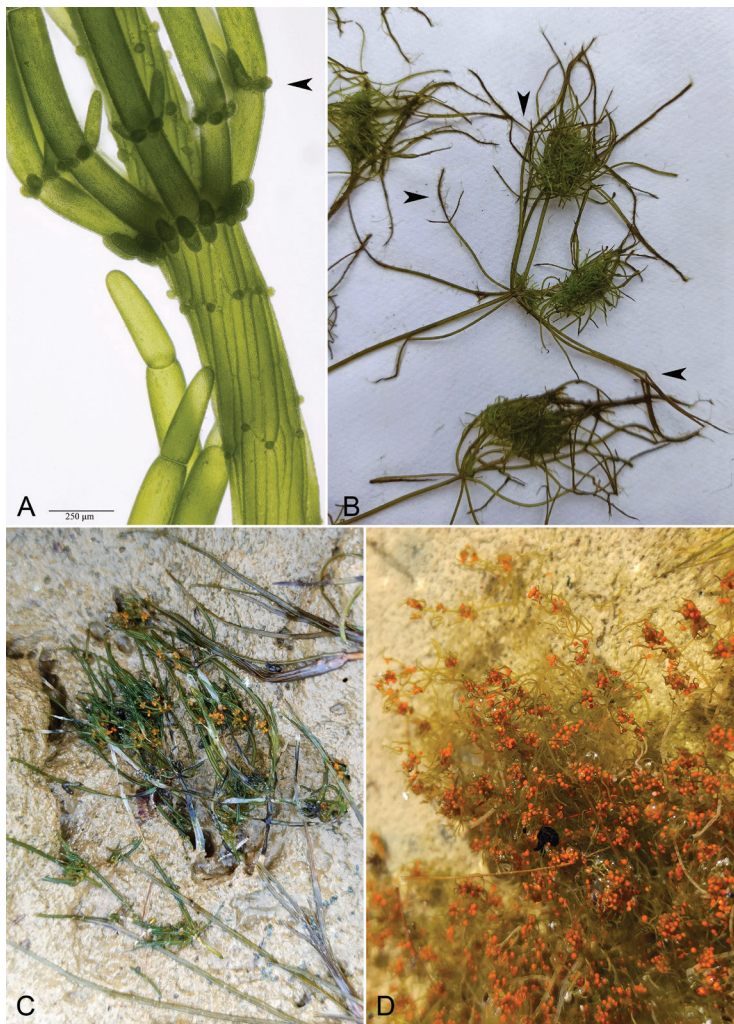
(IUCN criteria A1, B1, 2a,b, C1). It was recognized as “Critically Endangered” in the Red List of Balkan charophytes (Blaženčić et al., 2006b) and “Least Concern” in the provisional European Red List (Stewart, 2024).

In Southeast Europe, *Chara galioides* is known from very few sites in Albania (Zeneli & Kashta, 2016; Kashta et al., 2023) and Greece (Raabe & Koumpli-Sovantzi, 2000, 2002) (Figure 2b). It is distributed in coastal regions of Mediterranean mostly, in Europe and North Africa, as well as in Atlantic coast of France, Iberian Peninsula, and North Black Sea region, but most records are known from Western Mediterranean (Muller et al., 2017; Lambert & Schubert, 2024a and references herein).

***Chara squamosa*** Desf. (Figure 3a), Montenegro, Bar Municipality, southern slope of Rumija Mountain Ridge: 1. Vicinity of Tuđemili, well Manduke, at ca. 40 cm depth, 42.14605 N 19.13615 E, 10.02.2023. 2. North of Stari Bar, small stream – a left tributary of Zeljeznica

River, 42.14984 N 19.11771 E, 22.05.2023. Bijelo Polje Municipality: 3. Šipovica, small stream below the memorial fountain in water of Čamil Sijarić, 43.01773 N 19.89482 E, 08.07.2024. 4. Vojan grab, small stream, 43.02098 N 19.85268 E, 08.07.2024. Šavnik Municipality: 5. Bare, small stream near local road, 42.93076 N 19.25131 E, 15.08.2021.

Cyprus. 1. Agios Minas Chapel, west of Neo Chorio, Paphos district: small stream below spring near a road, at 1–3 cm depth, pH 8.01, electrical conductivity (EC) 880  $\mu\text{S}/\text{cm}$ , total dissolved solids (TDS) 625 ppm, salinity 0.40 ppt, 35.02400 N, 32.34152 E, 23.10.2024 (<https://www.inaturalist.org/observations/249076970>). 2. West of Ineia, Paphos district: Paphos, concrete basin, at 50–100 cm depth, pH 7.35, EC 1233  $\mu\text{S}/\text{cm}$ , TDS 868 ppm, salinity 0.60 ppt, 34.95555 N, 32.38611 E, 23.10.2024 (<https://www.inaturalist.org/observations/249076974>).



**Figure 3:** Key traits and the general habit of species from Cyprus and Montenegro. A – *Chara squamosa* from Cyprus: diplostichous tylacanthous stem cortex with short solitary spine cells, ecorticate branchlets (arrowhead), B – upper part of *Sphaerochara intricata*, arrowheads indicate typical forked sterile branchlets, C – general habit of *Tolypella nidifica*, D – general habit of male plants of *Tolypella hispanica* with large orange antheridia. Photographs: A – F. Bednár, B–D – R. E. Romanov.

**Slika 3:** Ključni znaci in splošen habitus vrst s Cipra in Črne gore. A – *Chara squamosa* s Cipra: dvoslojna grbančasta skorja stebila s kratkimi, posamičnimi trnskimi celicami, vejice brez skorje (puščica), B – zgornji del vrste *Sphaerochara intricata*, puščice kažejo značilne viličaste sterilne vejice, C – splošen habitus vrste *Tolypella nidifica*, D – habitus moške rastline vrste *Tolypella hispanica* z velikimi oranžnimi anteridiji. Fotografije: A – F. Bednár, B–D – R. E. Romanov.

This species is associated with small fresh-water mountain streams and artificial basins filled with fresh water in regions with limestone outcrops. The records from small water bodies can be evidence of the susceptibility of this species to interannual differences in weather and climate change threatening groundwater discharge. This species can be recognized as “Data Deficient” because its distribution in Montenegro and Cyprus is poorly known. It was not found among specimens from Cyprus collected by other botanists despite the targeted search in B,

L, LE, and W. It was recognized as “Critically Endangered” in Serbia (Trbojević et al., 2024) and in the Red List of Balkan charophytes (as *Chara conimbrigenensis* A. G. Cunha; Blaženčić et al., 2006b) and “Data Deficient” in the provisional European Red List (Stewart, 2024).

In Southeast Europe, *Chara squamosa* is known from Croatia, Serbia and North Macedonia (Figure 2c; Blaženčić & Blaženčić, 1997; Blaženčić et al., 2006b; Trbojević et al., 2024). It is distributed mostly in the Mediterranean. *Chara squamosa* is known from Europe, North Africa, and West Asia, including Caucasus (Romanov et al., 2023, 2024a and references herein). Considering its close similarity to *Chara gymnophylla* A. Braun, targeted checking of collections and new field studies can change a suggestion about the rare occurrence of this species in the south of Europe (Romanov et al., 2024a), especially in Southeast Europe, as it was recently shown for Serbia (Trbojević et al., 2024). However, this seems not to be the case for the Montenegrin Adriatic coast where it appears to be a rare species found in mountain landscapes only (this research). The same environment is notable for new sites from the Dinaric Alps in Montenegro and two locations from Cyprus (this research). The general occurrence of *Chara squamosa* in mountainous and hilly landscapes was found in Serbia and West Asia – Caucasus and Eastern Mediterranean (Trbojević et al., 2024; Romanov et al., unpubl. data). Few records of *Chara squamosa* from the Italian peninsula, Sicily, and the Balearic Islands fit the same pattern (Romanov et al., 2019, 2024a; Romanov et al., unpubl. data). Considering the re-identification of this species from Sardinia this distributional pattern seems suitable for this island too (cf. Becker, 2019; Romanov et al., 2024a). Therefore, more sites of *Chara squamosa* could be expected in the inland part of Montenegro, i.e. in the Dinaric Alps. This suggestion is not supported by the rare occurrence of *Chara gymnophylla*, the most similar to and not easily delineable with *Chara squamosa* (cf. Romanov et al., 2024a), in the country (Blaženčić & Stevanović, 2015; unpublished data by the authors; NHMM, checked by the authors). Genetic studies are implemented for this species group clarification (Romanov et al., in progress).

**Taxon of uncertain affiliation “Mediterranean *Chara*”** (Figure 1b), Montenegro, 1–6. Ulcinj Municipality, northeast vicinities of Velika plaža, inundated sand pits, 41.89706 N 19.29504 E, 41.89740 N 19.29045 E, 41.90015 N 19.27985 E, 41.89807 N 19.28941 E, 41.89703 N 19.29504 E, 41.89739 N 19.29044 E, 27.10.2022. 7–14. Budva Municipality, plain coastal area southern of Buljarica, brackish artificial permanent ponds and channels of the drainage system, at 20–50 cm depth,

42.18494 N 18.97176 E, 42.18490 N 18.97163 E, 42.18455 N 18.97165 E, 42.18668 N 18.97121 E, 42.18762 N 18.97092 E, 42.18560 N 18.97092 E, 42.18611 N 18.97170 E, 42.18671 N 18.97132 E, 24.06.2023.

This species is annual in Montenegro. The plants start germination in November becoming well-developed during June to October. The occurrence in only shallow water bodies can be evidence of the susceptibility of this species to interannual differences in weather and climate change. The urbanization of the coastal area in the vicinities of Buljarica and Velika plaža touching the existing drainage system and water bodies can be recognized as a threatening factor. This species can be referred to “Vulnerable” (IUCN criteria B2a,b, C2a, D2) in Montenegro. It was recognized as “Critically Endangered” in the Red List of Balkan charophytes (as *C. baltica* (Hartm.) Bruzelius; Blaženčić et al., 2006b) and “Data Deficient” in the provisional European Red List (Stewart, 2024)

“Mediterranean *Chara*”, a member of the subsection *Hartmania* R.D. Wood, or, in other words, a part of long-lasting “*botanicorum crux et scandalum*” considering still debatable species concepts, was tentatively accepted as a species in the monograph “Charophytes of Europe” (Blindow et al., 2024a). It requires further studies for evaluation. The Montenegrin plants are largely useful for this purpose. Some of them have apical parts characteristic for *Chara corfuensis*. However, they were growing in stands of plants mostly suitable for “Mediterranean *Chara*” leaving a space for doubts in their species identity. Probably, these taxa are conspecific and *Chara corfuensis*, which has a nomenclatural priority, represents a long-spiny, long-bracteate, and long-stipulate morphotype with verticillate bract cells. *Chara corfuensis* grows at good insolation without any restraints for the expression of the great length of bract cells and spine cells. Most spiny parts of plants are spotted near the surface of the water after the water level drops. It could be hypothesized that both insolation and, less probably, salinity increase can be responsible for this expression of morphological traits, essential for species delineation in the subsection *Hartmania* (see Blindow et al., 2024b). In Southeast Europe “Mediterranean *Chara*” was found in Slovenia, Albania, and Greece only; it was known there as *Chara baltica* (Hartm.) Bruzelius (Figure 2d; Firbas & Al-Sabti, 1995; Zeneli & Kashta, 2016; Blindow et al., 2024a). This taxon occurs mostly in the Mediterranean, but most records are available from the Western Mediterranean. It is known from the Atlantic coast of France too (Blindow et al., 2024a).

*Sphaerochara intricata* (Trentep. ex Roth) Feist-Castel & N. Grambast (Figure 3b), Montenegro, Ulcinj Municipality, vicinity of Fraskanjel, the easternmost part of Knetë Fraskanjel, temporary pond, depth 40–50 cm, 41.96580 N 19.37476 E, 02.04.2023.

The annual population appearing during spring only was found in the single plain freshwater shallow temporary water body existing during winter and spring in the depression inundated with waters of Lake Šas (Šasko jezero) and probably Bojana River. It is dry during summer and autumn. The finding in the temporary pond can be evidence of the susceptibility of this species to interannual differences in weather and climate change. This species can be referred to “Critically Endangered” (IUCN criteria B2a,b, C2a, D) in Montenegro. It was recognized as “Endangered” in the Red List of Balkan charophytes (as *Tolypella intricata* (Trentep. ex Roth) Leonh.; Blaženčić et al., 2006b) and “Vulnerable” in the provisional European Red List (Stewart, 2024).

This is a sparsely distributed rare species in Southeast Europe (Figure 3f), where it is known from Greece, Bulgaria, Serbia, and Romania (Blaženčić & Stanković, 2009; Vesić et al., 2011; Marković et al., 2023; van de Weyer, 2024). *Sphaerochara intricata* is a Holarctic species; its distribution area covers Eurasia, North Africa, and North America (Pérez et al., 2014; Korsh, 2018).

*Tolypella hispanica* Nordst. ex Allen (Figure 3d), Montenegro, Ulcinj Municipality, Nature Park “Ulcinjjska Solana”, brackish temporary ponds in abandoned crystallization and evaporation basins, and channel at few to 15 cm depths or spreading at wet clayish soil in the terrestrial environment after water evaporation: 1–8. 41.9235 N 19.2718 E, 17.04.2023; 41.92088 N 19.26240 E, 28.04.2023; 41.91960 N 19.25330 E, 41.91860 N 19.25270 E, 41.91860 N 19.25270 E, 30.04.2023; 41.92040 N 19.25210 E, 41.9109 N 19.3095 E, 06.05.2023; 41.91079 N 19.31099 E, 23.05.2023.

Greece. 1. Attica: in aquis salsuginosis stagnatibus, ad radices m. Kerata, prope Eleusin. 18 April 1886. De Helldreich herbarium graecum normale. Det.: 998. *Tolypella prolifera* (Ziz, Al. Br. Sub *Chara*) Leonh., Sydow Charae. P. 37, Nym. Consp. 879, No. 2, forma *typica*, fide Sydow! In litt. 1887. Det. U. Raabe: *Tolypella hispanica* 12.01.2000 (W 544). The same label. Det. R.E. Romanov (B 39755). The same label. Det. R.E. Romanov (LE). 2. Griechenland: in Graben im Phaleron bei Athen, 19 April 1911, Schniffer, Algae marinae No. 1319. Det. Schiffner: *Tolypella nidifica* (Müll.) Leonh. Det. U. Raabe: *Tolypella hispanica* 08.01.1999. Conf. R.E. Romanov (B 38624).

Annual populations appearing during only winter and spring were spotted in several plain brackish shallow temporary water bodies within Ulcinjska Solana only. The occurrence in only temporary ponds can be evidence of its high susceptibility to interannual differences in weather and climate change. This species can be referred to “Critically Endangered” (IUCN criteria B2a,b, C2a, D2) in Montenegro and Greece. It was recognized as “Critically Endangered” in the Red List of Balkan charophytes (Blaženčić et al., 2006b) and “Least Concern” in the provisional European Red List (Stewart, 2024).

The checking of old herbarium specimens stored in B, LE, and W and recent observations at iNaturalist.org (see below) allowed adding a few sites for Greece missed on the last map of species distribution in Europe (Troia et al., 2024). The part of old collection from Kerata, Greece, stored in Stockholm was reported as *Tolypella hispanica* by Horn af Rantzen (1959). In Southeast Europe, *Tolypella hispanica* is known from Croatia, Albania, and Greece (Figure 2e; Lovrić et al., 1989; Blaženčić et al., 1998; Troia et al., 2024; <https://www.inaturalist.org/observations/111904610>; <https://www.inaturalist.org/observations/111515902>; <https://www.inaturalist.org/observations/111516902>). It is distributed in Mediterranean Europe and North Africa (Muller et al., 2017; Troia et al., 2024 and reference herein), but most records are known from the Western Mediterranean. The remote records from West Siberia, Iran, and Pakistan (Groves, 1924; Romanov, 2019) can belong to the recently described vicariant species, *Tolypella mongolica* R.E. Romanov, V.S. Vishnyakov, V.Yu. Nikulin et A.A. Gontcharov (Romanov et al., 2024b).

*Tolypella nidifica* (O. F. Müll.) A. Braun (Figure 3c), Montenegro, Ulcinj Municipality, Nature Park “Ulcinjjska Solana”, brackish temporary ponds in abandoned crystallization basins, at the depth up to 10 cm and spread over wet clayish soil in a terrestrial environment after water evaporation: 1–3. 41.92731 N 19.27498 E, 41.92656 N 19.27403 E, 12.04.2023; 41.92088 N 19.26240 E, 28.04.2023.

Annual populations of *Tolypella nidifica* appearing during spring only were spotted in a few plain brackish shallow temporary water bodies in Ulcinjska Solana. The occurrence in only temporary ponds can be evidence of the high susceptibility of this species to interannual differences in weather and climate change. This species can be referred to “Critically Endangered” (IUCN criteria B2a,b, C2a, D) in Montenegro. It was recognized as “Critically Endangered” in the Red List of Balkan charo-

phytes (Blaženčić et al., 2006b) and “Least Concern” in the provisional European Red List (Stewart, 2024).

In Southeast Europe, *Tolypella nidifica* is known from a few records from Croatia only (Figure 2g; Visiani, 1842; Lovrić et al., 1989; Lambert & Schubert, 2024b). Some doubts are associated with the old record by Visiani (1842) because his specimen from the same location was later cited for *Nitella opaca* (C. Agardh ex Bruzelius) C. Agardh (Leonhardi, 1864). *Tolypella nidifica* occurs in coastal regions of Europe, but it has the main distribution center in the Baltic Sea region (Lambert & Schubert, 2024b and reference therein). North Africa, Caucasus, and Lake Issyk Kul in Kyrgyzstan harbor very few populations of *Tolypella nidifica* outside of Europe (Kausik & Bhattacharya, 1971; Hollerbach & Krassavina, 1983; Romanov et al., 2023; Romanov, unpubl. data).

## Conclusion

This research targeting all habitat types suitable for charophytes was implemented mostly in the Montenegrin Adriatic coast considering it was the least studied area of the country from a charophyte perspective (Blaženčić et al., 1998; Biberdžić et al., 2006; Biberdžić & Blaženčić, 2013; Blaženčić & Stevanović, 2015; Blaženčić et al., 2018; Biberdžić et al., 2023). It resulted in new national records of seven species: *Chara corfuensis*, *Chara galioides*, *Chara squamosa*, *Sphaerochara intricata*, *Tolypella hispanica*, *Tolypella nidifica* and “Mediterranean *Chara*”, mostly typical for the Mediterranean and rare outside this region. *Chara squamosa* was found for the first time in Cyprus. The checking of herbarium collections revealed one new national record for Croatia, *Chara galioides*, and two old neglected sites of *Chara galioides* and *Tolypella hispanica* for Greece. In Montenegro, all species were found only in the southern part in plain temporal and permanent small water bodies, except for *Chara squamosa*, which was also found in the mountainous southern and northern regions in small water bodies. The habitats of all species fit the environment known to them (Schubert et al., 2024). New locations enriched charophyte species distribution data for Southeast Europe adding new sites of regionally rare species. They were used in the tentative assessment of species protection statuses. They will be helpful for future

evaluation of important charophyte areas. However, the territory of the Nature Park “Ulcinjaska Solana”, the easternmost vicinities of Velika plaža, and the coastal wetland southern of Buljarica can already be recognized as important charophyte areas in Montenegro and Southeast Europe. The updated species list of Montenegrin charophytes counts 37 species making Montenegro one of the charophyte species-rich countries in Europe.

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