

Article

Checklist, Red List, and Distribution Pattern of Charophytes (Charophyceae, Charales) in Slovakia Based on Critical Revision of Herbarium Specimens

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Abstract: A critical revision of charophytes collected in Slovakia from 1887 up to present, based on herbaria specimens, provided us with the opportunity to compile an updated checklist and to reevaluate their threat status in order to obtain the current red list of charophytes of Slovakia. The data obtained will fill the gap in knowledge about these macroscopic algae in Central Europe. The revised list of charophytes includes 19 representatives of three genera: *Chara* (11), *Nitella* (7), and *Nitellopsis* (1). Following the latest IUCN red-list categories and criteria (version 3.1) and using the modified category regionally extinct (RE), derived from the category extinct (EX), 36.8% of charophytes are classified as RE (*Chara connivens*, *C. subspinosus* (=rudis), *Nitella capillaris*, *N. confervacea*, *N. flexilis*, *N. gracilis*, and *N. opaca*); 21% as EN (endangered; *Chara braunii*, *Nitella mucronata*, *N. syncarpa*, and *Nitellopsis obtusa*); 21% as VU (vulnerable; *Chara contraria*, *C. gymnophylla*, *C. hispida*, and *C. virgata*); 10.6% as NT (near-threatened; *Chara globularis*, *C. vulgaris*); and 5.3% as CR (critically endangered; *Chara canescens*) species; finally, 5.3% species could not be assessed (DD; *Chara aspera*).

Keywords: *Chara*; *Nitella*; *Nitellopsis*; Slovakia; IUCN categories



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1. Introduction

Charophytes, commonly known as stoneworts, include six living genera that have been assigned, based on morphological criteria, to two tribes within the family Characeae: the tribe Chareae includes four genera (*Chara* L., *Lamprothamnium* J. Groves, *Nitellopsis* Hy, *Lychnothamnus* (Ruprecht) A. Braun) and the tribe Nitelleae two genera (*Nitella* C. Agardh and *Tolypella* (A. Braun) A. Braun [1]). The taxonomy of this group of algae has been problematic and remains unresolved in many respects. Taxonomic classification is still being revised not only at the level of species but also of genera, mainly according to molecular analyses, e.g., some current phylogenetic studies indicate that *Lamprothamnium* can be a part of the genus *Chara*. On the other hand, *Tolypella* was split into two genera: *Tolypella* and *Sphaerochara*. This is related to the fact that the genus *Sphaerochara* Mädlar was already included in the family Charophyceae, based also on the morphological characteristics of the oospores and gyrogonites, e.g., [2,3].

With the exception of Antarctica, charophytes have spread to all continents [4,5]. In Europe, more than 60 species have been recorded so far [5–7]. Knowledge about their distribution varies from country to country, mainly due to the focus of the research, the intensity and scope of the charophyte observations, including the teamwork capacity and the significant differences in data collection, including with regard to herbarium sheets and/or the management of herbaria. Countries with an intensive and long-term charophyte research, e.g., in Great Britain, Netherlands, Germany, Poland, and the Balkan

or Nordic countries, have relatively comprehensive information on the species richness of charophytes, their distribution, and their ecological demands [7–14].

Stoneworts prefer freshwater, less brackish and saline environments, and natural or man-made habitats, and they are able to colonise newly created habitats as pioneers e.g., [11,15]. They are found in both lotic and lentic habitats covering a broad scale of habitat types [2,16,17]. Charophytes grow in waters that are relatively clear/ transparent, acidic to alkaline, shallow to deep (up to several ten meters), and oligotrophic to mesotrophic, and some species are able to tolerate eutrophic polluted waters [2,5,9,13,16]. In addition to local ecological variables, a set of regional ecological characteristics such as altitude, latitude, and climatic factors also play an important role in the distribution pattern, richness, and abundance of these algae [13,18–20].

Charophytes are endangered in several European countries, from the western to the eastern part [6,8,21,22], due to their specific ecological requirements; their strong human pressure on freshwater habitats; and, especially, their weak competitiveness in aquatic vegetation.

Slovakia is one of the countries with a relatively poor knowledge of charophytes. The data are scattered in several, mainly local contributions published only sporadically, e.g., [23–29]. The exception is aquatic vegetation with charophytes as dominant plants, which was recently rather comprehensively summarized by Hrivnák et al. [30].

The reasons for compiling both a new checklist and a new red list of charophytes in Slovakia was the fact that 20 years had passed since the publication of both lists [31,32] and new data on charophytes had been published in the meantime. However, a critical revision of the specimens was still missing. We therefore proceeded to revise two Central European herbaria (one stored in Bratislava, Slovakia and the other one in Poznań, Poland) with numerous sheets of charophytes collected in the territory of Slovakia since 1887 to 2020, and we managed to evaluate the current conservation status of these algae.

2. Materials and Methods

The basic source of this study was two herbarium collections of macroscopic algae of order Charales sampled from different parts of Slovakia: the main collection is part of the Herbarium of the Institute of Botany, Plant Science, and Biodiversity Center of the Slovak Academy of Sciences (SAS) Bratislava, Slovakia, and the second collection is in the Herbarium of the Natural History Collections Faculty, Adam Mickiewicz University (POZG) in Poznań, Poland. A total of 253 herbarium specimens collected independently by 32 collectors during the years 1887–2020 were critically revised. The highest number of specimens (125) was collected in the last two decades (2000–2019), mainly by H. Oľahel'ová, R. Hrivnák, K. Bubíková, and A. Hindáková from Slovakia, and in 1959 with 59 specimens by I. Dąmbska from Poland (Figure 1).

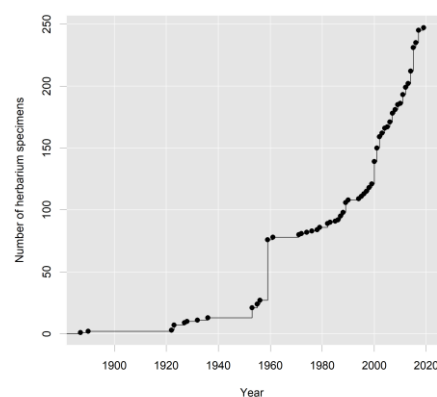


Figure 1. Temporal pattern in herbarium specimen collection.

A new grid system of the Atlas Florae Europaeae (further on AFE; <https://www.luomus.fi/en/new-grid-system-atlas-florae-europaeae> (accessed on 15 September 2022)) was used to determine the distribution pattern of the studied charophytes in Slovakia.

The IUCN red list Categories were applied in the present red list using the IUCN guidelines [33–35]. The IUCN criteria A–F were used to identify the categories for each species [33]. Our evaluation was done (i) strictly based on herbarium data and, in the case of generally rare stoneworts, (ii) the revision of old localities during last decade.

The identification of species written on the herbarium labels and those collected from the field was verified according to Krause [5] and Urbaniak and Gąbka [11]. The nomenclature of charophytes follows the AlgaeBase [3]. All taxa mentioned in the text or summarized in Table 1 are listed at least the first time with the full name of the author together with the year of its description.

Table 1. Checklist and red list of charophytes in Slovakia with the number of its determined herbarium specimens and grid cell occurrence. Species signed as 1 and 2 were mentioned in previous Check List (1: Hindák and Hindáková 1998) and red list (2: Hindák and Hindáková 2001). No. sp. = number of specimens, No. gc. = number of grid cells.

Species Name	No. sp.	No. gc.	IUCN Category
^{1,2} <i>Chara vulgaris</i> Linnaeus 1753	115	21	NT
^{1,2} <i>Chara globularis</i> Thuiller 1799	42	12	NT
^{1,2} <i>Chara contraria</i> A.Braun ex Kützing 1845	27	9	VU (B2c, C2b)
^{1,2} <i>Chara hispida</i> Linnaeus 1753	23	7	VU (B2c, C2b)
<i>Chara virgata</i> Kützing 1834	15	9	VU (B2c, C2b)
¹ <i>Chara gymnophylla</i> A.Braun 1835	8	5	VU (B2a,c, C2b)
^{1,2} <i>Nitellopsis obtusa</i> (N.A.Desvaux) J.Groves 1919	7	2	EN (B2a,c; C2ai,b)
² <i>Nitella mucronata</i> (A.Braun) F.Miquel in H.C.Hall 1840	6	5	EN (B2a,c; C2ai,b)
² <i>Chara braunii</i> C.C.Gmelin 1826	4	4	EN (B2a,c; C2ai,b)
¹ <i>Nitella gracilis</i> (J.E.Smith) C.Agardh 1824	3	2	RE
¹ <i>Chara subspinoso</i> Ruprecht 1846 (= <i>Chara rudis</i> (A.Braun) Leonhardi 1863)	2	1	RE
^{1,2} <i>Nitella flexilis</i> (Linnaeus) C.Agardh 1824	2	2	RE
^{1,2} <i>Nitella syncarpa</i> (J.L.Thuillier) Kützing 1845	2	1	EN (B2a,c; C2ai,b)
<i>Nitella capillaris</i> (A.J.Krocker) J.Groves and G.R.Bullock-Webster 1920	2	2	RE
^{1,2} <i>Chara canescens</i> Loiseleur 1810	1	1	CR (B1a, 2a)
<i>Chara connivens</i> P.Salzmann ex A.Braun 1835	1	1	RE
² <i>Nitella confervacea</i> (Brébisson) A.Braun ex Leonhardi 1863	1	1	RE
^{1,2} <i>Nitella opaca</i> (C.Agardh ex Bruzelius) C.Agardh 1824	1	1	RE
^{1,2} <i>Chara aspera</i> Willdenow 1809	.	.	DD

3. Results

3.1. Revision of Herbarium Specimens, Checklist, and Distribution Pattern of Charophytes

In Slovakia, altogether 19 species from three genera, *Chara* (11), *Nitella* (7), and *Nitellopsis* (1), were identified from herbarium sheets (Table 1 and Figure 2). Specimens of the *Chara vulgaris* were the most frequent within the studied herbaria, followed by *C. globularis*, *C. contraria*, *C. hispida*, and *C. virgata*. Almost 18% and more than 23% species were documented by only one or two herbarium specimens, respectively (Table 1).

According to these data, stoneworts were recorded in almost three quarters of the territory of Slovakia, and they were present in ~73% of grid cells. The highest species richness was found in the southwest in the lowland Podunajská nížina (6–9 species in ~8% grids), while average (3–5 species) and low (1–2 species) richness was found in 30% and 35% of grid cells, respectively (Figure 3).

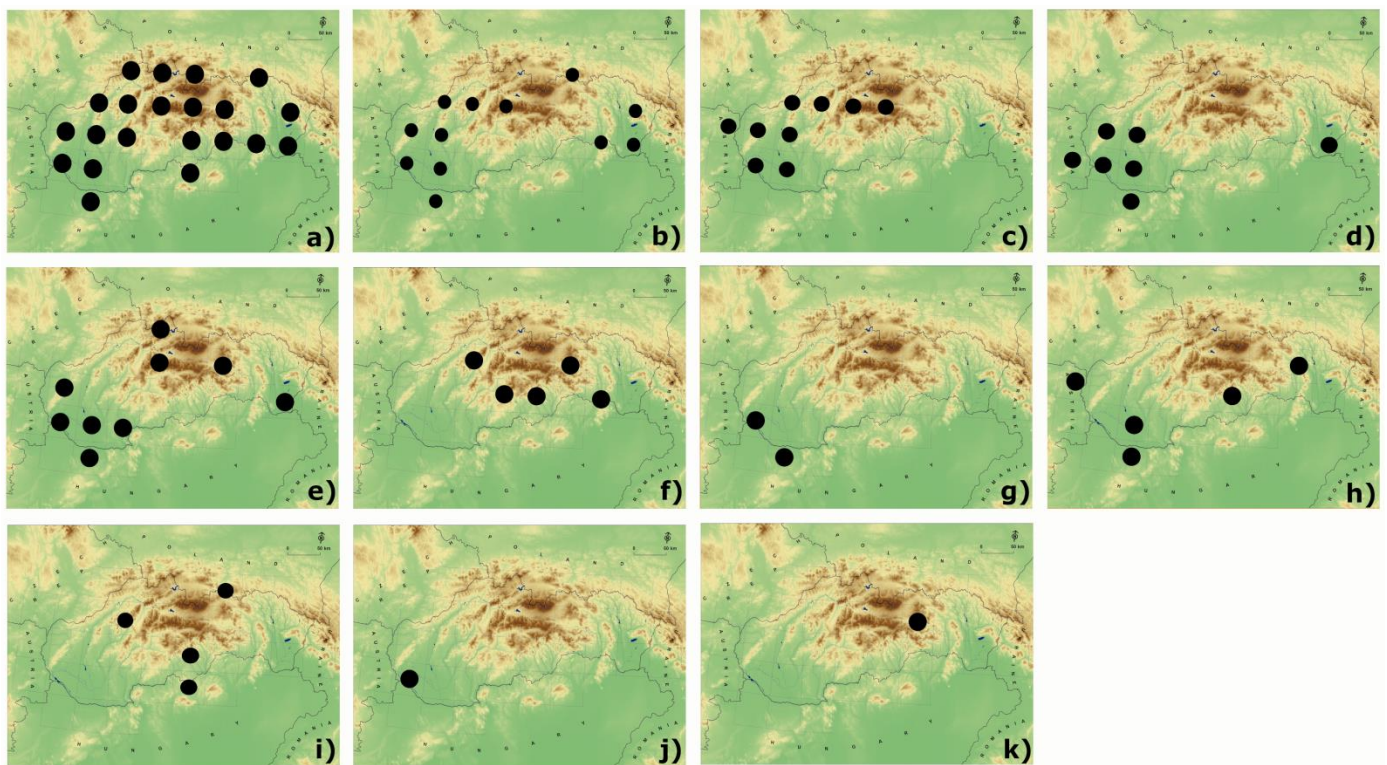


Figure 2. Distribution of individual charophytes in Slovakia (only species with the IUCN categories NT, VU, EN, and CR are listed) in AFE grid cells. (a) *Chara vulgaris*, (b) *C. globularis*, (c) *C. contraria*, (d) *C. hispida*, (e) *C. virgata*, (f) *C. gymnophylla*, (g) *Nitellopsis obtusa*, (h) *Nitella mucronata*, (i) *C. braunii*, (j) *Nitella syncarpa*, and (k) *C. canescens*.

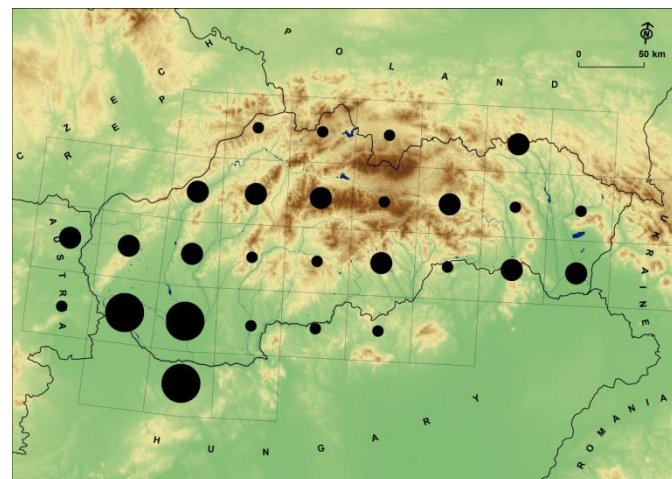


Figure 3. Distribution of charophytes in Slovakia: species richness in AFE grid cells (• 1–2 species, • 3–5 species, and • 6–9 species).

3.2. Red List of Charophytes

The presented red list includes seven taxa assessed as regionally extinct (RE), one critically endangered species (CR), four endangered species (EN), four vulnerable species (VU), two species in the category near-threatened (NT), and that one could not be evaluated due to insufficient data (DD). The highest number of charophytes is classified as strictly endangered (CR, EN, and VU, ~47%), followed by RE (~37%) and near-threatened taxa (~10%; Table 1).

4. Discussion

4.1. Changes in Lists of Charophytes

The authors of the last checklist of non-vascular and vascular plants of Slovakia [31] reported sixteen species of charophytes from the genera *Chara*, *Nitella*, *Nitellopsis*, and *Tolypella*. These, together with later published records of *Nitella translucens* and *N. mucronata* [26], were evaluated in the red list of charophytes in Slovakia [32]. The total number of known species from all available publications up to the present day is eighteen. This number appears to be small, but it should be noted that some species show great intraspecific morphological variability. In the past, these variations were the reason for the description of new species; today, they are reclassified as forms or varieties of one species [3,7].

In the present study, we are presenting nineteen species of charophytes. There are several reasons for the different numbers of species recorded, for example, different taxonomic concepts, new findings from the last twenty years, critical revisions of herbaria specimens by various specialists, or the type of studied source (all published data were used in the previous checklist, while only herbaria specimens were taken into account in this study).

4.2. Comments to Newly Recognized and Undetermined Species in Slovakia

A critical revision of herbarium specimens revealed charophytes that have not been registered in Slovakia before: *Chara connivens*, *C. virgata*, *Nitella capillaris*, and *N. confervacea* are new for the Slovak flora. *Chara virgata* was arranged in the past according to the rank of the variety, namely, as “*virgata*” of the species *C. globularis* [5], and this variety was not recognized in the material of charophytes in Slovakia. *Chara virgata* is a relatively widespread stonewort in northern Europe [11,13]; therefore, the numerous findings in herbaria specimens in Slovakia, after critical revision, are not surprising. Stoneworts *Chara connivens* and *Nitella confervacea* were collected by S. Hejný from rice fields near Kolárovo in 1953 and 1955, respectively (rice fields disappeared within a few years due to different agricultural management). These historical findings in southern Slovakia remain interesting as these stoneworts occur mainly in the Mediterranean area and several Nordic European countries [3,7] and, except for Hungary, are considered relatively rare in Central Europe. Caisová and Gabka [2] have already used these old herbarium sheets (POZG) to complete a survey on their European distribution. However, the occurrence of *C. connivens* has not been confirmed since then, so it is considered as regionally extinct (RE) in Slovakia. In Europe, *Nitella confervacea* has been recorded from many countries, but it appears to be extremely rare in much of its range [3]. Due to its small size (it is the smallest of the charophytes and is very difficult to find in the field), this species requires regular disturbance of its habitat. The species *Nitella capillaris* sampled in the Podunajská and Borská nížina lowland in 2002 by H. Oľahel'ová and T. Kiss (SAV herbarium) was correctly determined by H. Oľahel'ová, but these findings have never been published. The species range of *Nitella capillaris* is confirmed from the neighbouring countries, Hungary, the Czech Republic, or Poland [2,5,11]. From eight sites, the occurrence of *C. gymnohylla*, a species rare in Central Europe, was documented in herbarium materials. A typical habitat for this species is aquifer springs, including thermal and mineral springs. The species is critically endangered in more European countries [2]. On the contrary, the species of the three genera *Chara*, *Nitella*, and *Tolypella* from the previous checklist were not confirmed among the revised herbarium sheets: *Chara aspera* Willdenow; *C. pistianensis* J. Vilhelm; *C. scepusiensis* N. Filarszky; *N. translucens* (Persoon) C. Agardh; and *Tolypella prolifera* (Ziz ex A.Braun) Leonhardi. Information on the occurrence of *Chara aspera* in Slovakia comes from Bratislava and Spiš see [36]. Later findings are not documented by herbarium sheets including our herbaria material. In addition, only general information without the exact location of the species occurrence is mentioned from Slovakia [37,38]. *Chara scepusiensis* recorded in the Tatra National Park [23] is currently considered as a form of *C. contraria* (valid as *C. contraria* f. *scepusiensis*) [3]. Like *C. pistianensis*, this stonewort, which was collected by J. Vilhelm and J. Bílý in Piešťany in 1922 (and 1932, respectively, POZG) and described in 1924 by the first author as a new species, is currently regarded only as a form of *C. vulgaris* [3]. Specimens of

Nitella translucens published from two localities in the southern part of central Slovakia have not been correctly identified [26]. As the revision of herbarium sheets showed, the correct determination is *Chara braunii*. Similarly, the specimens of *Tolypella prolifera* collected from fishponds near Bohel'ov (the Podunajská nížina lowland, southern Slovakia; O'ahel'ová 1987 SAV) were re-determined as *Chara hispida* after critical revision.

4.3. Comments to Critically Endangered and Some Regional Extinct Species

Only one species, *Chara canescens*, represents critically endangered species in Slovakia. In 2020, a few individuals were found in samples from shallow-water depressions near a recent travertine hill in the Spiš region (northeastern Slovakia). This species can be found around Europe, but its occurrence outside the sea coast is limited in inland areas [7]. In Slovakia, the occurrence of this species from Spiš is known from the past (1926). Although this stonewort is currently known in only one locality and we do not know the total size of the populations, we assume that it has a chance to survive as the individuals found were in good condition and fertile [39].

Altogether, seven charophyte species were classified as regionally extinct in our red list (Table 1). Especially, *Chara connivens*, as a rare species in Central Europe, is often found in shallow saltwaters from south-western Europe up to the Baltic Sea and coastal lakes [3]. So far, there is only one record from Slovakia from the 1960s, namely, from rice fields cultivated in the vicinity of Komárno. *Nitella opaca*, as a robust stonewort, is common in many European regions, preferring deep lakes [40]. In the past, it was found in an arm of the river Váh in 1887 near Trenčín (Holuby, POZ), and it has not been found since then.

4.4. Threat Status of the Stoneworts in Slovakia: Comparison of Previous and New Red Lists

The basis of any evaluation of living organisms is a correct determination of taxa, which also requires sufficient experience in understanding their morphological variability. Charophytes are characterized by enormous intraspecific variability, resulting in numerous descriptions of forms, a number of synonyms, and still unresolved taxonomic issues [3,5]. Some experience with collecting charophytes at the appropriate time (when individuals are well developed and fertile), well-preserved herbarium specimens that are as numerous as possible and taxonomists with many years of experience are important for successful identification.

The compilation of a red list of charophytes is possible if an adequate number of collections is available over a relatively long period (at least for more than half a century). In Slovakia, we are only approaching such ideal conditions for the evaluation of charophyte populations. The authors of the contribution on cyanobacteria and algae in the Conservation of Flora in Slovak Republic [41] have already stated that the findings of more than 50 species and varieties are only historical data, see [36].

The first red list for Slovakia was published as The Red Book of Threatened and Rare Species of the Slovak and Czech Republics [42]. From each taxonomic group, a small number of species was selected from the most endangered localities, such as swamps, peat bogs, and wet meadows. They serve as examples that generally represent a certain model for the protection of these algae. Among the charophytes, only five species threatened in Slovakia were selected and classified as endangered or critically endangered. The second version of the red list of cyanophytes/cyanobacteria and algae of Slovakia [32] included mainly taxa described from our territory or those whose discovery in Slovakia represents extremely valuable information about the existence of these organisms in Europe or in the world in general. A total of 15 charophytes were presented and uniformly evaluated as endangered (EN) in the red-list categories. Both red lists of Slovakia [32,42] reflect negative changes in the populations of charophytes, leading to a direct threat caused by various factors, mostly human activities. The authors have already pointed out that the only effective and possible way to protect species is through the protection of their habitats and the global protection of the environment [32,41,42]. Along with the destruction and/or degradation of habitats, unique species are being lost, and the gene pool is irreversibly

impoverished [41]. In our updated red list, there are 19 species of charophytes that have been critically revised on the basis of two herbarium collections and subsequently classified into different endangered species categories. We used the latest IUCN criteria [33,34], a different approach from the previous ones, so a direct comparison to the previous lists is practically impossible.

We are aware that further information, especially habitat and population characteristics, and/or a revision of all historical sites of stoneworts and the research of the potential sites of the species, would be useful in the assessment of individual species. However, this may be the subject of further research.

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