

# Records of new algal taxa within various aquatic and aerophytic habitats in Slovenia

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**Abstract.** In Slovenia, the first algal research dates back to the year 1845, when Kützing referred to several localities in Slovenian territory in his work »Phycologia germanica«. The present knowledge of diversity, variety and abundance of algae, however, is still incomplete, which applies both to aquatic and terrestrial species. The presented study carried out in 2005 and 2006 included 30 different aquatic and 5 terrestrial environments. It embraced data on species composition and distribution of benthic species in aquatic ecosystems and terrestrial environments. 634 taxa from ten classes were identified, 170 of which were discovered in Slovenia for the very first time. Most new identified taxa in Slovenia belong to Bacillariophyceae, specifically *Navicula* and *Nitzschia*. The genera of *Amphidinium*, *Bumilleria*, *Heterothrix*, *Stylopyxis*, *Entomoneis*, *Stenopterobia*, *Chlorophysema*, *Podohedra* and *Poloidion* had not been recorded in Slovenia prior to this study.

Key words: benthic algae, aerophytic algae, springs, waterfalls, oxbow lakes, brackish waters, Slovenia

**NOVI TAKSONI ALG V RAZLIČNIH VODNIH IN AEROFITSKIH HABITATIH V SLOVENIJI** – Prve raziskave alg na območju Slovenije segajo v leto 1845, ko je Kützing v svojem delu »Phycologia germanica« omenil tudi naše kraje. Poznavanje vrstne strukture, pestrosti in abundance alg pa je še vedno nepopolno. To velja tako za vodne kot kopenske vrste. Predstavljene sezonske raziskave v letih 2005 in 2006 vključujejo 30 različnih vodnih okolij in 5 terestričnih. Vključujejo podatke o vrstni sestavi in razširjenosti bentoških vrst v vodnih ekosistemih in terestričnih okoljih. Evidentirali smo 634 taksonov iz desetih razredov, od tega 170 taksonov prvič na ozemlju Slovenije. Največ na novo ugotovljenih taksonov v Sloveniji pripada razredu kremenastih alg in sicer rodovoma *Navicula* in *Nitzschia*. Rodovi *Amphidinium*, *Bumilleria*, *Heterothrix*, *Stylopyxis*, *Entomoneis*, *Stenopterobia*, *Chlorophysema*, *Podohedra* in *Poloidion* pa do sedaj niso bili znani v Sloveniji.

Ključne besede: bentoške alge, aerofitske alge, izviri, slapovi, mrtvice, brakične vode, Slovenija

## Introduction

At present, algology is a deficient sub-discipline of biology and ecology in Slovenia. There are many reasons for this state of affairs, including the fact that today the research is largely dedicated to polluted aquatic ecosystems (lakes, dams, rivers), mainly as monitoring of potential contamination or changes in trophicity. The algae of distinct (including extreme) environments, such as peat bogs, fens, marshy meadows, thermal springs, pools, brackish waters, soil, humid rocks, rock walls, stumps, tree trunks, etc., are particularly poorly investigated (Krivograd Klemenčič 2007).

A better knowledge of algal diversity is important in the processes of natural heritage conservation. Algae are key indicators of anthropogenic pressures on aquatic ecosystems and mineralization level. The presence, absence and abundance of algae are the parameters for the assessment of conditions of aquatic ecosystems (Wetzel & Likens 1995). The importance of algae is even greater in extreme conditions, such as blooming lakes (Sedmak & Kosi 1997).

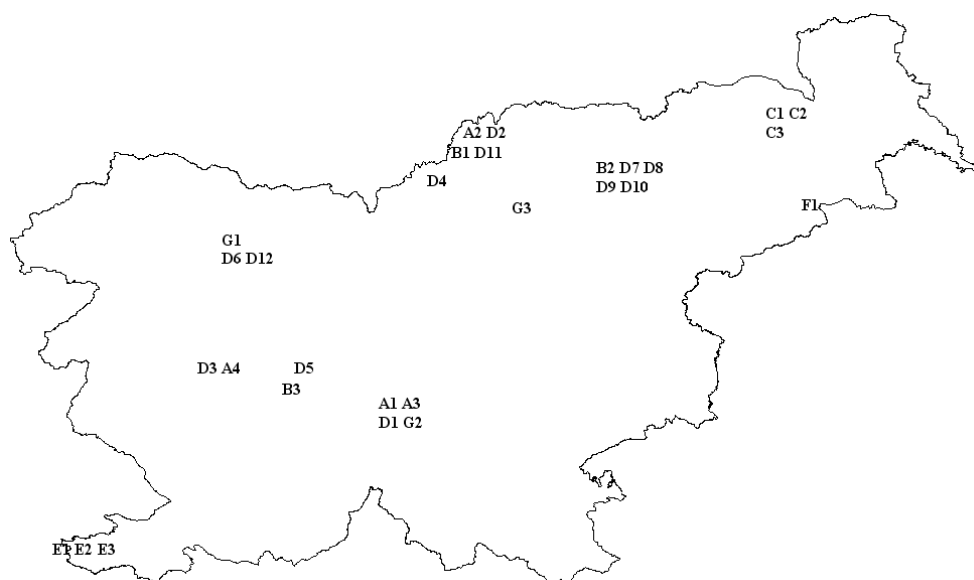
Stevenson (1996) estimates that about 26,000 algal species inhabit inland waters. In Slovenia, 2,067 species of algae and cyanobacteria have been identified so far (Vrhovšek et al. 2006). The data indicate great diversity of aquatic ecosystems; however, it is difficult to verify this number, considering that many of the localities of aquatic ecosystems studied in the past do not exist anymore or have been severely altered or polluted (Vrhovšek et al. 2006). At the same time, the new recorded species increase this number.

In Slovenia, the study of algae started in 1845, when the world-famous algologist Kützing referred to a few localities from Slovenian territory in his book »Phycologia germanica« (Kützing 1845). Some other well-known algologists completed his work: Grunow, Hansgirg, Pascher, Keissler and Pevalek (Vrhovšek et al. 2006). The first systematic algal research was conducted by Lazar (Lazar 1960, Lazar 1975). Until then, 512 algal species had been known (Vrhovšek et al. 2006). Lazar collected his research results in the book »Alge Slovenije« (Lazar 1960) and later in his book »Razširjenost sladkovodnih alg v Sloveniji« (Lazar 1975). After 1975, some individual algologists worked in Slovenia: Golubić, Munda, Vrhovšek and Kosi. The algae in karst caves have been studied in detail by Mulec (2005), Mulec et al. (2007, 2008), Mulec & Kosi 2008. However, the last comprehensive contribution on algae in Slovenia was the »Monograph on Freshwater and Terrestrial Algae in Slovenia« (Vrhovšek et al. 2006).

## Materials and methods

### Selection of sampling points

The Excel computer database of freshwater and terrestrial algae of the Limnos Company was the basis for the selection of 30 sampling sites in aquatic and 5 in terrestrial environments (Fig. 1, Tab. 1). The examined habitats presented in this study, previously algologically poorly examined, were springs, waterfalls, oxbows, bogs, mires, brackish waters and terrestrial aerophytic habitats. Additional criterion for the selection of sampling sites applied in this study was type of substratum on which algae were attached in aquatic environments, limestone, silicate, flysch and other substrates, such as decayed wood stump, limestone rocks, concrete and soil in terrestrial habitats.



**Figure 1.** Map of Slovenia with marked sampling sites. See Table 1 for sampling sites codes.

**Slika 1.** Zemljevid Slovenije z označenimi vzorčnimi mesti. Oznake vzorčnih mest so prikazane v Tabeli 1.

**Table 1.** Sampling sites with codes  
**Tabela 1.** Seznam vzorčnih mest z oznakami

Habitat	Code	Sampling site	Substratum with attached algae	Bedrock	No. of taxa
springs	A1	spring on Kopanj	concrete, wood, moss	limestone	26
	A2	spring on Koroški Selovec	stones, wood, moss	silicate	54
	A3.1	Šica spring - basin	rocks, pebbles, detritus	limestone	88
	A3.2	Šica spring - beginning of the water course	stones, wood, macrophytes	limestone	87
	A4	spring on Medvedje Brdo	moss	limestone	47
waterfalls	B1	small waterfall on Navrški vrh	rocks, moss	limestone	37
	B2	waterfall on the Bistrica tributary on Pohorje	moss	silicate	94
	B3.1	waterfall near the path in the Pekel canyon at Borovnica	moss	limestone	28
	B3.2	the first waterfall in the Pekel canyon at Borovnica	rocks	limestone	52
	B3.3	the fifth waterfall in the Pekel canyon at Borovnica	rocks	limestone	37
oxbows	C1	sampling site river Mura oxbow – Zaton I	wood, macrophytes	silicate	37
	C2	sampling site river Mura oxbow – Zaton II	wood, detritus	silicate	75
	C3	sampling site river Mura oxbow – Mali Bakovci	wood, detritus, macrophytes	silicate	107
bogs, mires	D1	Radensko polje	macrophytes	limestone	84
	D2	bog at Koroški Selovec	macrophytes	silicate	79
	D3	Žejna dolina bog	moss	limestone	118
	D4	bog at Holmec	moss, macrophytes	silicate	59
	D5	Mali plac bog on Ljubljansko barje	wood, detritus, macrophytes	limestone	59
	D6	Ledina bog on Jelovica	moss, macrophytes	limestone	125
	D7	bog above the Tinčeva bajta on Pohorje	wood, moss, detritus, macrophytes	silicate	85
	D8	bog I on the way to Osankarica	moss	silicate	51
	D9	bog II on the way to Osankarica	wood, moss, detritus, macrophytes	silicate	84
	D10	Črno jezero on Pohorje	wood, moss, macrophytes	silicate	105
	D11	puddle on Navrški vrh	wood, silt	silicate	61
	D12	puddle on Jelovica	wood, stones, silt	limestone	56
brackish waters	E1	Fazana estuary	stones, concrete, macrophytes	flysch	60
	E2	Rižana estuary	stones, concrete, macrophytes	flysch	87
	E3.1	Roja estuary (I)	wood, macroalgae	flysch	97
	E3.2	Roja estuary (II)	macrophytes, detritus	flysch	81
constructed wetland	F1	Constructed wetland Dobrava near Ormož	macrophytes	silicate	14
aerophytic habitats	G1	pine stump on Jelovica	decayed wood stump	/	8
	G2	wet cliffs at the water course Šica spring	rocks, moss	limestone	51
	G3.1	Huda luknja cave entrance	rocks, moss	limestone	25
	G3.2	Huda luknja (soil at the cave entrance)	soil	limestone	12
	G3.3	abandoned railway tunnel near the Huda luknja cave	concrete walls, limestone rocks	/	28

## Sampling and identification of algae

A list of sampling sites is shown in Table 1. The samples were taken in 2005 and 2006. At least one but not more than four samples were collected at each sampling site. In total, 131 samples were taken at 35 sampling sites, 114 of which were taken from 30 sampling sites in aquatic environments and 17 from five terrestrial sampling sites. Only benthos algae were collected at the sampling sites in aquatic environments.

Algae in aquatic environments were sampled semiquantitatively. Phototrophs were taken randomly from different colonised substrata. In bogs, mires, oxbow lakes, springs, waterfalls and estuaries, scrapes of algal mat were sampled from the surface of pebbles, stones, rocks, macrophytes and submersed wood. Algae associated with mosses were sampled by squeezing the submerged talus of a moss (mires, e.g. a spring at Koroški Selovec, a spring at Medvedje Brdo, a waterfall on the Bistrica tributary). Samples of silt were taken from the bottom of both puddles. Water at the springs at Koroški Selovec and Kopanj is directed through a pipe into a wooden trough, which was scraped for floristic analysis (Tab. 1).

In aerophytic habitats, surfaces with algal mats were scraped using a sharp knife. Specimens were afterwards resuspended in a small quantity of distilled water.

Samples were preserved in the field with 35 % formaldehyde (Merck) with the final concentration of 4 %. Samples from bogs, mires and the constructed wetland were examined twice, prior to and after fixation with formaldehyde. Such samples often contain flagellates, which change their morphology to indistinguishable level when formaldehyde is applied (Wołowski & Hindák 2005).

Samples were examined in the laboratory using light microscopes Nikon Eclipse E400 and Nikon Eclipse TE300, equipped with digital camera Nikon Digital Camera DXM 1200, Japan. Images were processed using software Lucia 4.6 (Laboratory Imaging s.r.o., Czech Republic). In order to determine the diatoms to the level of species, the samples first had to be treated suitably with concentrated HNO<sub>3</sub> (Schaumburg et al. 2004). Permanent slides with diatoms were made from the treated samples by Naphrax (Schaumburg et al. 2004). Slides were examined under a magnification of 1000 and 1200×, and phase contrast microscopy was applied where necessary. Individual algal cells were photographed to observe cell structures in detail. Abundance of individual taxa was estimated using the method described by Grbović (1994) (Tab. 2).

**Table 2.** Scale for estimation of algal taxa abundance (Grbović 1994).**Preglednica 2.** Lestvica za ocenjevanje pogostosti posameznih taksonov alg (Grbović 1994).

<b>Abundance estimation</b>	<b>Taxa present in % of visual fields</b>
1 - single	1-15
3 - customary	>15-60
5 - dominate	>60-100

The following keys were used for algal identification: Cleve-Euler (1951, 1952, 1953a, b, 1955), Ettl (1978, 1983), Ettl & Gärtner (1988, 1995), Heering (1914), Hindák (1996, 2006), Hindák et al. (1978), Hortobágyi (1973), Komárek & Anagnostidis (1998, 2005), Krammer (2000), Krammer & Lange Bertalot (1997a, b, 2004a, b), Lazar (1960), Lenzenweger (1996, 1997, 1999, 2003), Popovsky & Pfiester (1990), Rieth (1980), Starmach (1968, 1972, 1974, 1977, 1980, 1983), Wołowski & Hindák (2005).

## Results and discussion

In the present study, 464 algal taxa were identified of a total of 2,067 taxa from the list of freshwater and terrestrial algae in Slovenia (Vrhovšek et al. 2006). 170 algal taxa were identified for the first time in Slovenia (Tabs. 3-4) (compare Vrhovšek et al. 2006). The majority of new identified taxa belonged to Bacillariophyceae (108), followed by Cyanobacteria (19), Chlorophyceae (15), Zygnematophyceae (16), Xanthophyceae (8), Euglenophyceae (2), Dinophyceae (1) and Chrysophyceae (1). Most new recorded taxa belonged to the *Navicula* (32) and *Nitzschia* genera (17). The genera of *Amphidinium*, *Bumilleria*, *Heterothrix*, *Stylopyxis*, *Entomoneis*, *Stenopterobia*, *Chlorophysema*, *Podohedra* and *Poloidion* were identified for the first time in Slovenia.

















**Table 4.** List of new recorded algal taxa in aerophytic environments in Slovenia. See Table 1 for sampling site codes.**Tabela 4.** Seznam novo ugotovljenih taksonov v aerofitskih okoljih za Slovenijo. Oznake vzorčnih mest so prikazane v Tabeli 1.

Taxa	Sampling sites				
	G1	G2	G3.1	G3.2	G3.3
<b>PROKARYOTA</b>					
<b>CYANOPHYTA</b>					
CYANOPHYCEAE					
<i>Gloeocapsa lignicola</i> Rabenhorst	•				
<i>Leptolyngbya gracillima</i> (Zopf ex Hansgirg)			•		
Anagnostidis & Komárek					
<i>Phormidium papyraceum</i> Gomont ex Gomont			•		•
<b>EUKARYOTA</b>					
<b>HETEROKONTOPHYTA</b>					
BACILLARIOPHYCEAE					
<i>Achnanthes kryophila</i> Petersen			•		•
<i>Amphora inariensis</i> Krammer		•			
<i>Cymbella similis</i> Krasske			•		
<i>Navicula insociabilis</i> Krasske			•	•	
<i>Navicula nivaloides</i> Bock			•		•
<i>Navicula soehrensensis</i> Krasske				•	
<i>Navicula tenelloides</i> Hustedt				•	
<i>Nitzschia alpina</i> Hustedt				•	•
<i>Orthoseira dendroteres</i> (Ehrenberg) Crawford			•		•
<i>Pinnularia intermedia</i> (Lagerstedt) Cleve			•		
<i>Pinnularia obscura</i> Krasske		•			
<i>Stauroneis obtusa</i> Lagerstedt			•		
<b>CHLOROPHYTA</b>					
CHLOROPHYCEAE					
<i>Coccomyxa confluens</i> (Kützing) Fott	•				
<i>Podohedra bicaudata</i> Geitler	•				
<i>Podohedra falcata</i> Düringer	•				
<i>Poloidion didymos</i> Pascher			•		
<i>Trentepohlia annulata</i> Brand	•				

## Springs

A total of 20 taxa were identified in four springs, which had not been registered before in Slovenia. 18 belonged to Bacillariophyceae and 2 to Xanthophyceae. Most of them were from the genera of *Achnanthes* (4), *Navicula* (4) and *Nitzschia* (4). Especially rich site regarding the »new« species was the spring of Šica (13), where both new species of the Xanthophyceae class (*Bumilleria spirotaenia* and *Heterothrix quadrata*) were recorded. *B. spirotaenia* occurs as green mat on silted bottoms of water bodies (Ettl 1978); something similar was observed at the sampling point in the Šica spring.

## Waterfalls

18 new taxa for Slovenia were identified in five waterfalls. Of the newly found taxa in Slovenia, 16 were from Bacillariophyceae, *Homoeothrix janthina* from Cyanobacteria and *Heterodendron squarrosom* from Xanthophyceae. Most new species belonged to the genera of *Navicula* (4) and *Achnanthes* (3).

## Oxbows

In the oxbows of the Mura River, a total of 25 taxa were identified, which had not been known before in Slovenia. Of the new taxa, 19 belonged to diatoms, *Borzia curta* and *Woronichinia robusta* to Cyanobacteria, *Bumilleria klebsiana* and *B. spirotaenia* to Xanthophyceae, while *Characium ensiforme* and *Microspora abbreviata* belonged to Chlorophyceae.

## Bogs and mires

Samples from Radensko polje contained 12 taxa, previously not identified in Slovenia. Eight new taxa belonged to diatoms, three to Chlorophyceae, and *Peroniella minuta* to Xanthophyceae.

In mires and in the lake of Črno jezero, 85 new taxa were identified, 44 of which belonged to Bacillariophyceae, 14 to Zygnematophyceae, 12 to Cyanophyceae, eight to Chlorophyceae, three to Xanthophyceae, two to Euglenophyceae, one to Dinophyceae, and one to Chrysophyceae. The most recently identified taxa belonged to the genera of *Navicula* (12), *Pinnularia* (9) and *Eunotia* (6). There were 15 new taxa for Slovenia identified in the bog at Koroški Selovec, 15 in the peat bog of Žejna dolina (Krivograd Klemenčič & Paradiž 2006), 11 in the peat bog at Holmec, six in the peat bog of Mali plac at the Ljubljana bog, 12 in the peat bog of Ledina, 27 in the lake of Črno jezero, 10 in the bog above the Tinče cottage, eight in the bog I on the way to Osankarica, and nine in the bog II on the way to Osankarica.

In puddles at Jelovica and Navrški vrh, nine taxa were identified that had not been recorded before in Slovenia. Four new taxa belonged to Chlorophyceae, three to Bacillariophyceae, and two to Zygnematophyceae.

## **Brackish water**

36 taxa were identified as new records in Slovenia. 33 new taxa belonged to diatoms and three to Cyanobacteria.

## **Constructed wetland**

14 taxa were identified (Krivograd Klemenčič 2008), but there were no new records for Slovenia.

## **Aerophytic habitats**

In total, 18 new taxa were recorded in aerophytic habitats, which have not been known until now in Slovenia. 12 taxa belonged to Bacillariophyceae, four to Chlorophyceae. *Gloeocapsa lignicola* and *Phormidium papyraceum* belonged to Cyanobacteria. The majority of identified species were typical aerophytes. Especially rich in »new« species were humid rocks at the entrance to the cave of Huda luknja. At this sampling site, a green alga *Poloidion didymos* was identified, which is the only representative of the *Poloidion* genus often inhabiting humid soils and mosses from the liverworts group (Ettl & Gärtner 1995). The other three species of the Chlorophyceae class (*Podohedra bicaudata*, *P. falcata* and *Trentepohlia annulata*) were identified on a pine stump on the Jelovica plateau. All three species are rather common in Europe, but they were identified for the first time in Slovenia, because this habitat (tree stumps) had simply been overlooked in previous studies.



## Reasons for the high number of new recorded taxa in Slovenia

1. Poor algal research in the territory of Slovenia. In Slovenia, algae have been studied systematically only by Lazar (1975). After 1975, the studies of few algologists have been limited mainly to polluted watercourses and lakes (retarding basins). Many other habitats have been studied only by Krivograd Klemenčič (2001) within the framework of her master's thesis. In his paper, Lazar (1975) presented thoroughly the classes of Chlorophyceae, Zygnematophyceae, Chrysophyceae and Euglenophyceae. To a lesser extent, he also studied Cyanophyceae, Chrysophyceae and Xanthophyceae. Regarding Bacillariophyceae, Lazar (1975) wrote: »The data on diatoms are very poor because I limited myself only to those most common and frequent and to those which I was able to determine without losing much time.« It can be therefore assumed that the above mentioned statement was one of the reasons why the majority of new identified taxa within our research were from the group of diatoms, and less from other algal groups.

2. Overlooked habitats. The largest number (29) of the new recorded algal taxa was identified in the outfall of the Roja watercourse. Brackish waters in Slovenia are algologically poorly studied. Prior to this research, the only study of algae in brackish waters of Slovenia was carried out at the outfall of the Dragonja River (Krivograd Klemenčič et al. 2003), in which the authors identified 38 algal taxa, unknown until then in the territory of Slovenia. In addition to freshwater and brackish algae, marine species can be found in brackish waters, which are carried to the mouth of watercourses by tide. There are some data on macrobenthic algae in the Slovenian sea (Matjašič & Štirn 1975; Battelli 1998, 2002), but no information on the species composition of microbenthic algae. Therefore, the marine algal species identified in brackish waters (*Leptolyngbya jadertina*, *Oscillatoria margaritifera*, *Cocconeis scutellum*, *Diploneis didyma*, etc.), with the exception of macrobenthic green alga *Enteromorpha intestinalis*, were recorded as new species in Slovenia. Interestingly high number of the new identified taxa (28) was recorded in the lake of Črno jezero at Pohorje, which is the only lake with low pH in Slovenia and as such represents a habitat for many algal species that do not thrive in other parts of Slovenia. The lake Črno jezero at Pohorje had not been algologically researched prior to this study. Moreover, raised bogs, fens, marshes, pools and terrestrial aerophytic habitats have been poorly researched in Slovenia as well. For example, of the eight species on a pine stump at Jelovica four had not been previously recorded in Slovenia. Among them was a green alga *Trentepohlia annulata*, which can be easily observed macroscopically as an orange mat on the surface of pine stumps.

3. Inadvertence of particular algal species. The authors may have incorrectly identified many species of algae, in particular the small ones (under 10  $\mu\text{m}$ ). They might have overlooked them or even lost them due to an unsuitable sampling procedure. In this study, the scanning electronic microscopy (SEM) was not used for identification of diatoms owing to the problems connected with SEM sample preparation. Specifically, most samples were taken from stagnant water from a siliceous substratum, which contained much organic material. This material cannot be removed in the preparation of diatom samples by concentrated  $\text{HNO}_3$  to the extent that enabled the visibility of the cell surface structure by SEM. It is reasonable to assume that by the application of a more suitable procedure for the preparation of diatoms for SEM and subsequent identification of algae by the means of SEM, a much larger number of new records of diatoms would have been identified.

## Povzetek

Prve raziskave alg na območju Slovenije segajo že v leto 1845, ko je Kützing v svojem delu »Phycologia germanica« omenil tudi naše kraje. Poznavanje vrstne strukture, pestrosti in abundance alg pa je še vedno nepopolno. To velja tako za vodne kot aerofitske vrste. Predstavljene sezonske raziskave v letih 2005 in 2006 vključujejo 30 različnih vodnih okolij in 5 terestričnih okolij. Vključujejo podatke o vrstni sestavi in razširjenosti bentoških vrst v vodnih ekosistemih in terestričnih okoljih. Evidentirali smo 464 od skupno 2067 taksonov, do zdaj znanih za Slovenijo, in 170 taksonov, ki doslej še niso bili zabeleženi na našem ozemlju. Največ za Slovenijo novih taksonov pripada razredu kremenastih alg (108), in sicer rodovoma *Navicula* (32) in *Nitzschia* (17), sledijo Cyanobacteria z 19, Chlorophyceae s 15, Zygnematophyceae s 16, Xanthophyceae z osmimi, Euglenophyceae z dvema, Dinophyceae z enim in Chrysophyceae prav tako z enim taksonom. Rodovi *Amphidinium*, *Bumilleria*, *Heterothrix*, *Stylopyxis*, *Entomoneis*, *Stenopterobia*, *Chlorophysema*, *Podohedra* in *Poloidium* so bili v Sloveniji ugotovljeni prvič. V izvrih smo ugotovili 20, v slapovih 18, v mrtvicah reke Mure 25, v barjih in močvirjih 106, v brakičnih vodah 36 ter v aerofitskih habitatih 18 za Slovenijo novih taksonov alg. V rastlinski čistilni napravi Dobrova pri Ormožu nismo ugotovili za Slovenijo novih taksonov alg. Razlogi za tako visoko število novo ugotovljenih taksonov alg so 1) nizka stopnja raziskanosti alg na območju Slovenije, 2) neraziskanost izbranih habitatov in 3) prezrtost določenih vrst alg.

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