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The phytocoenology, distribution, and habitat preferences of the species *Aldrovanda vesiculosa* (Droseraceae) in Romania

Simona Dumitrița Chirilă[1], Mihai Doroftei[1*], Kiril Vassilev[2] and Silviu Covaliov[1]

1 Danube Delta National Institute for Research and Development, Babadag Street no. 165, 820112 Tulcea, Romania
2 Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Gagarin Street 2, 1113 Sofia, Bulgaria
* Correspondence: mihai.doroftei@ddni.ro

ABSTRACT:
Aldrovanda vesiculosa is an endangered aquatic species in Romania which grows in shallow waters where competition with other aquatic species is limited or absent. A comprehensive overview of the species' distribution and habitat preferences in Romania is currently lacking. In this context, this study aimed to update the distribution and habitat preferences of the species. Localities from Dobrogea, Oltenia, Muntenia, Transylvania, and Crișana represented the study areas. For the vegetation classification, 71 relevés with *A. vesiculosa* were analysed. The size of the sample areas ranged from 4 m^2 to 25 m^2. Vegetation syntaxonomic assignment was performed by agglomerative hierarchical clustering. The optimal number of clusters was chosen using the corrected Rand and Silhouette indices. Diagnostic species were identified for each cluster based on the indicator value index (IndVal). The study results showed that the dominant majority of the previous populations of *A. vesiculosa* (89%) were no longer identified from Romania in the 2008–2021 period. Recently (2021–2023), they were found only in six localities in the Dobrogea, Transylvania, and Oltenia regions. The causes of the disappearance of the species are eutrophication and the lowering of the water level. From a phytocoenological point of view, 11 plant communities were identified where *A. vesiculosa* is found. According to the EUNIS classification, this species inhabits wetlands (Q) and inland surface waters (C). In conclusion, translocation is suggested as the primary conservation measure for protecting the species.

Keywords:
habitat characteristics, phytocoenology, recent distribution, translocation, waterwheel

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INTRODUCTION

Aquatic ecosystems are among the most threatened ecosystems on Earth (Dudgeon 2019; Ferreira *et al.* 2023). They face multiple threats, including land-use change, environmental pollution, water drainage, and the added pressure of global climate change (Adamec 1999; Poff *et al.* 2002; Arthington *et al.* 2016). One of the critical issues is the eutrophication of wetlands, which is a significant threat to aquatic biodiversity worldwide (Cross *et al.* 2020). Extensive agricultural practices in Europe have harmed these ecosystems, causing the deforestation of numerous habitats for aquatic species and contributing to eutrophication (Adamec 2018; Cross *et al.* 2020). Ensuring the preservation and protection of these invaluable ecosystems is of the utmost importance, mainly by land use and water level management.

Carnivorous plants are particularly threatened by the clearing of natural habitats for agricultural and residential development, pollution, and invasive species (Brewer & Schlauer 2018; Cross *et al.* 2020). It was estimated that by 2050, carnivorous plants are likely to lose 100% of their potential range (Fitzpatrick & Ellison 2018). The most significant pressure is habitat loss (Thompson

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et al. 2017; Staude *et al.* 2018; Cross *et al.* 2020). Over time, some studies (Cross 2012; Cross *et al.* 2015, 2016; Adamec 2018; Cross & Adamec 2020) have focused on identifying conservation measures for *Aldrovanda vesiculosa* L.. Introducing the species into potentially favourable areas is the most appropriate conservation measure for this species (Cross & Adamec 2020).

Aldrovanda vesiculosa, commonly known as the waterwheel, is one of the aquatic species threatened by eutrophication (Adamec 2018). It is a relict species (Yakubovskaya 1991; Adamec 2018; Płachno *et al.* 2020), hydrophyte and perennial geophyte (Bilz & Lansdown 2011) from the family *Droseraceae* Salisb. (Jury 2009). The current species' name was given by Carl Linné (Linné 1753), whereas its description was provided by Charles Darwin (Darwin 1875).

It is native to nutrient-poor oligo-mesotrophic and dystrophic habitats (Adamec 1995a). It occupies a narrow ecological niche with specific habitat requirements (Cross & Adamec 2020). The species is found in various habitats such as lakes, water storage, ponds, pools in peat bogs, stagnant water pools, large river basins, lagoons (Adamec 2018; Cross & Adamec 2020), lake margins (Beldie *et al.* 1955), shallow lakes, peaty lakes and pools, marshes, external reed belts (Lamont *et al.* 2013) as well as mesotrophic lakes and eutrophic habitats (Breckpot 1997; Adamec 1999). Moreover, *A. vesiculosa* is a stenotopic and carnivorous species (Cross & Adamec 2020), which gives it a reduced competitive ability and limits the species distribution to microhabitats dominated by a low number of species (Kaminski 1987; Adamec 1995b, 2018). These microhabitats consist of shallow waters, where competition with other aquatic species is limited or absent (Kaminski *et al.* 1996; Cross & Adamec 2020). In addition, the species prefers habitats with clean, warm, standing, and intense light, with low concentrations of nutrients and a slightly low pH (Adamec & Kovářová 2006). *Aldrovanda vesiculosa* can also be considered a bioindicator of wetland health (Cross 2012).

Currently, the species distribution area is highly restricted due to anthropogenic factors. Primarily, this species is threatened by residential and commercial development, agriculture and aquaculture, changes in natural systems, and pollution (Jennings & Rohr 2011; Clarke *et al.* 2018). Other threats to the species include acidification, channelisation, siltation, drainage, eutrophication, deforestation, gravel extraction, mining, pollution, hydrological alteration, and limited dispersal (Commission of the European Communities 2009; Bilz & Lansdown 2011). According to Adamec (2018), the main threats to the species are eutrophication and decreasing water levels. Another threat is represented by illegal trade (Cross & Adamec 2020). The species' distribution is facilitated by birds and water currents (Beldie *et al.* 1955; Cross 2012). Consequently, the species' demise is linked to its profound susceptibility to habitat degradation (Kaminski 1987). A disorder was observed in the *A. vesiculosa* shoot apices, manifesting as the yellowing, then blackening, and finally, the death of the shoot apices. Termed *Aldrovanda* disease, this disorder is caused by the fungal pathogen belonging to the genera *Phytopythium* and *Pythium* (Oomycetes class). This disease hinders the growth of apical shoots, causing their growth to slow down and eventually stop (Svobodová & Adamec 2020).

The distribution of the species shows that it has been reported in Europe, Asia, Africa, and Australia (Atsuzawa *et al.* 2020). Nevertheless, the history of is colonisation has not been clarified (Płachno *et al.* 2020). Thus, some studies (Huber *et al.* 1961; Sculthorpe 1967) mention that *A. vesiculosa* survived the glaciations in Africa and Australia and later recolonised Europe, while others (Cross 2012) suggest that *A. vesiculosa* survived glaciations in southern European refugia and colonised Africa and Australia approximately 50,000–200,000 years ago. Thus, further studies are needed to clarify the origin of this species. Although the species has a wide distribution range, populations have declined in all areas of the world (Nishihara *et al.* 2023). Presently, the species shows a highly fragmented distribution (Cross & Adamec 2020). In Europe, it has been reported from southern Scandinavia to the south of France and east through the Balkans to Ukraine (Bilz & Lansdown 2011). Its distribution spans a vast geographical expanse, encompassing Kazakhstan and India in Asia, traversing the Primorskye and Amur regions of the Russian Far East, China, Japan, and the Korean Peninsula, extending to the S Sahara and Madagascar in Africa, and reaching NW and SE Australia (Bilz & Lansdown 2011; Płachno *et al.* 2020). It has been introduced into various ponds in North America, where it is thought to become potentially invasive (Lamont *et al.* 2013; Płachno *et al.* 2020).

According to Cross & Adamec (2020), *A. vesiculosa* has been reported in 28 sites in Asia (one extant population, seven unverified populations, and 20 extinct populations), 28 sites in Australia (six extant populations, and 22 unverified populations), 298 sites in Europe (43 present populations, 88 unverified populations and 167 extinct populations), and 38 sites in Africa (seven extant populations, five verified populations and 26 unverified populations).

At the global level, the species is endangered (Cross & Adamec 2020; EEA 2023), and at the European and European Union levels, the species is classified as being data deficient (Bilz & Lansdown 2011; EEA 2023). For Romania, the species is classified as critically endangered (Dihoru & Negrean 2009) or endangered (Oltean *et al.* 1994; Oprea 2005). With the species' decline, measures have been taken to protect it through both national and international legislation. Thus, the species

Fig. 1. *Aldrovanda vesiculosa* L. A – the vegetative stage; B – the flowering stage. (Photos: S. Covaliov, July 2023)

is mentioned in the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention 1979 – annex I) and in the Habitats Directive (EU Habitats Directive - annexes II, IV). Although conservation measures have not been effective (Cross & Adamec 2020), the best measures are the introduction of populations into potentially suitable areas (Adamec 2018; Cross & Adamec 2020). About 87% (329 "world" populations) have not been confirmed in the last century (Cross & Adamec 2020).

Efforts to prevent species extinction necessitate a comprehensive understanding of their distribution, ecological requirements, and the threats they face. In certain instances, species can be effectively conserved by translocating or relocating them to suitable habitats devoid of the pressures which endanger them in their original range. Translocation involves directly transferring individuals of a species to a new area (Liu *et al.* 2015), while relocation entails introducing individuals reared outside their natural habitat, such as botanical gardens, into a new location within the vicinity of their existing population.

This study aimed to conduct a distribution and phytocoenological analysis of *A. vesiculosa* in Romania. The objectives of the study were: (i) updating the distribution of the species in Romania; (ii) identifying the phytocoenological characteristics of *A. vesiculosa* communities, and (iii) recommending conservation measures for the protection of the species in Romania.

MATERIAL AND METHODS

Study species. *Aldrovanda vesiculosa* (Fig. 1) is an aquatic, rootless perennial species (Beldie *et al.* 1955; Nishihara *et al.* 2023). The stem is simple, thin, weakly branched, green, or brown (Beldie *et al.* 1955; Nishihara *et al.* 2023). The leaves grow in a whorl of eight and end in traps (Beldie *et al.* 1955; Pogorzelec *et al.* 2022). Each leaf has two lobes connected to the midrib (Atsuzawa *et al.* 2020). The flowers are white, small, axillary, and solitary (Beldie *et al.* 1955; Cross 2012), supported by short peduncles above the water (Cross *et al.* 2012). *Aldrovanda* flowering is rare (Adamec & Tichý 1997; Adamec 2018). As a frost survival strategy, *A. vesiculosa* forms turions. The growing season of the species is from early spring to mid-autumn (Adamec 2018). The leaves and petiole cells contain abundant chloroplasts (Atsuzawa *et al.* 2020).

Study area. The study was conducted in the Dobrogea, Oltenia, Muntenia, Transylvania, and Crișana regions (Romania). The climate is temperate-continental, with annual mean precipitation from 300 mm to 600 mm and annual mean temperatures from 10°C to 13°C.

Species distribution. A comprehensive literature review, including management plans and forms for Natura 2000 sites (Anonymous 2015, 2016a, b, 2020a, b, 2023), articles and books (Oprea 2005; Dihoru & Negrean 2009; Irimia & Bartók 2017) and specialised databases, as well as authors' field observations, was undertaken to gather comprehensive species distribution data. In this particular context, the information documented in the literature and gathered through fieldwork was arranged into maps using QGIS version 3.28 (QGIS Development Team 2022).

Vegetation sampling and classification. For the vegetation analysis, 71 relevés with *A. vesiculosa* (including

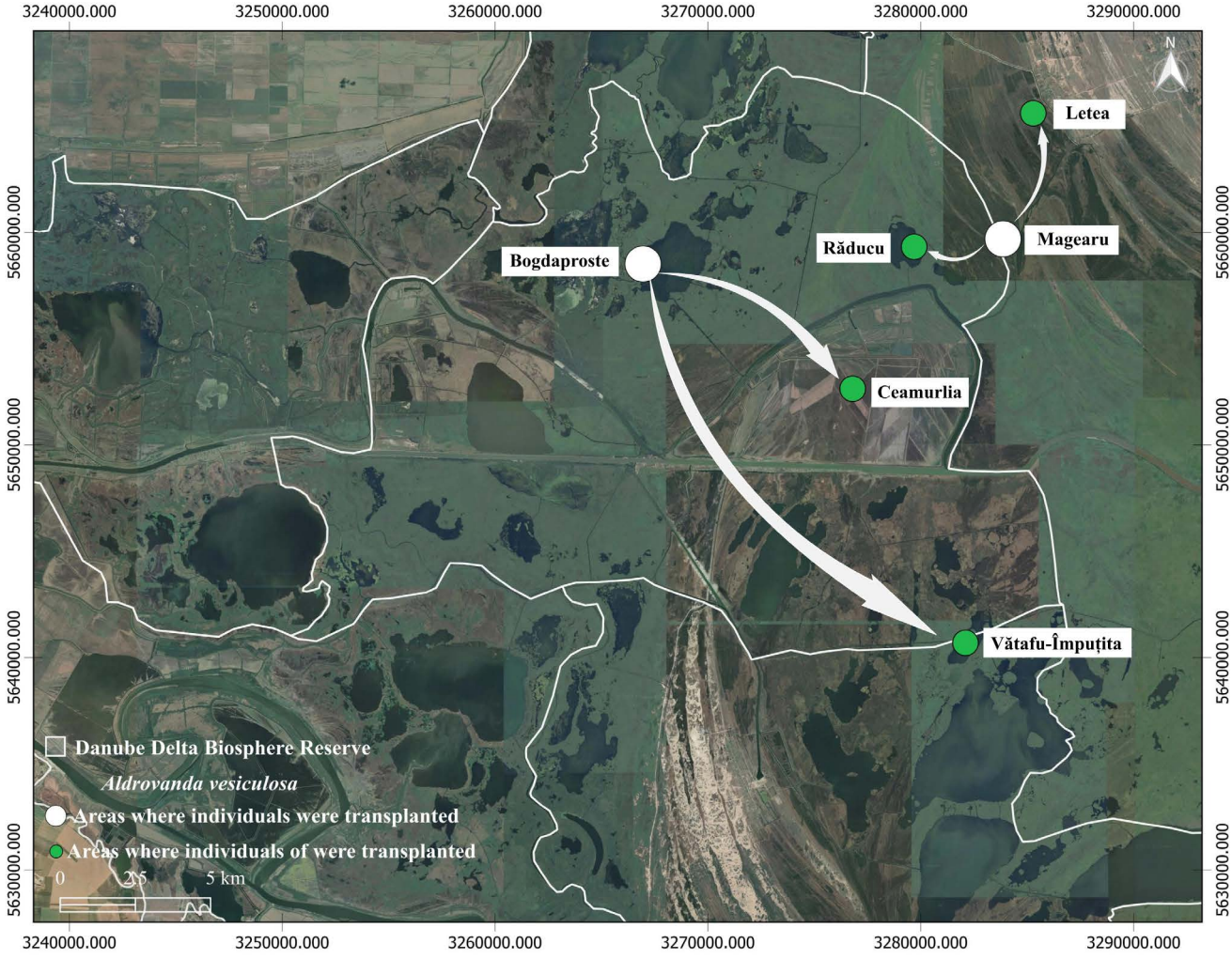

Fig. 2. The areas of *Aldrovanda vesiculosa* individual transplantation. (Map: S.D. Chirilă)

75 taxa) were used: 21 relevés obtained from the Romanian Grasslands Database (RGD; EU-RO-008; Vassilev *et al*. 2018), made between 1959–2008; and 50 relevés collected by fieldwork from April to July 2008–2022 carried out using the transect method. The size of the plots was as follows: ranging from 4 m^2 to 25 m^2 for the RGD plots; and 25 m^2 for personal plots. The vegetation was classified using the hierarchical agglomerative grouping method (minkowski method and the Bray-Curtis dissimilarity), using the mean of the Braun-Blanquet coverage-abundance scale, adapted to the vegetation in Romania (Borza & Boşcaiu 1965; Cristea *et al*. 2004): r (0.05%); + (0.5%); 1 (5%); 2 (17.5%); 3 (37.5%); 4 (62.5%); 5 (87.5%).

The optimal number of clusters was chosen using the Adjusted Rand Index (Rand 1971) and the mean Silhouette index (Rousseeuw 1987). The dendrogram was made in the R program version 5.1, using the packages "dendextend" (Galili 2015), "tidyverse" (Wickham *et al*. 2019), and "cluster" (Maechler *et al*. 2022). Diagnostic species were identified for each cluster based on the indicator value index (IndVal; Dufrêne & Legendre 1997). This index was validated by a permutation test (de Cáceres & Legendre 2009) using the "multipatt" package in R Studio version 5.1 (RStudio Team 2023). The synoptic table was made using JUICE 7.1 software (Tichý 2002). The nomenclature of the vegetation followed the specialised literature: the plant species follows Euro+Med (2023), the plant associations follow Coldea *et al*. (2017), and higher syntaxonomists follow Mucina *et al*. (2016). The classification of habitats was carried out according to EUNIS (Chytrý *et al*. 2020).

Species translocation. The activity was carried out in the Danube Delta from 2016 to 2022, from the end of May to the beginning of August. The source plants had dimensions in the range from 8 cm to 17 cm with a single branch viable for developing turions. The individuals were manipulated in the morning when the temperatures were lower. The specimens were moved from the

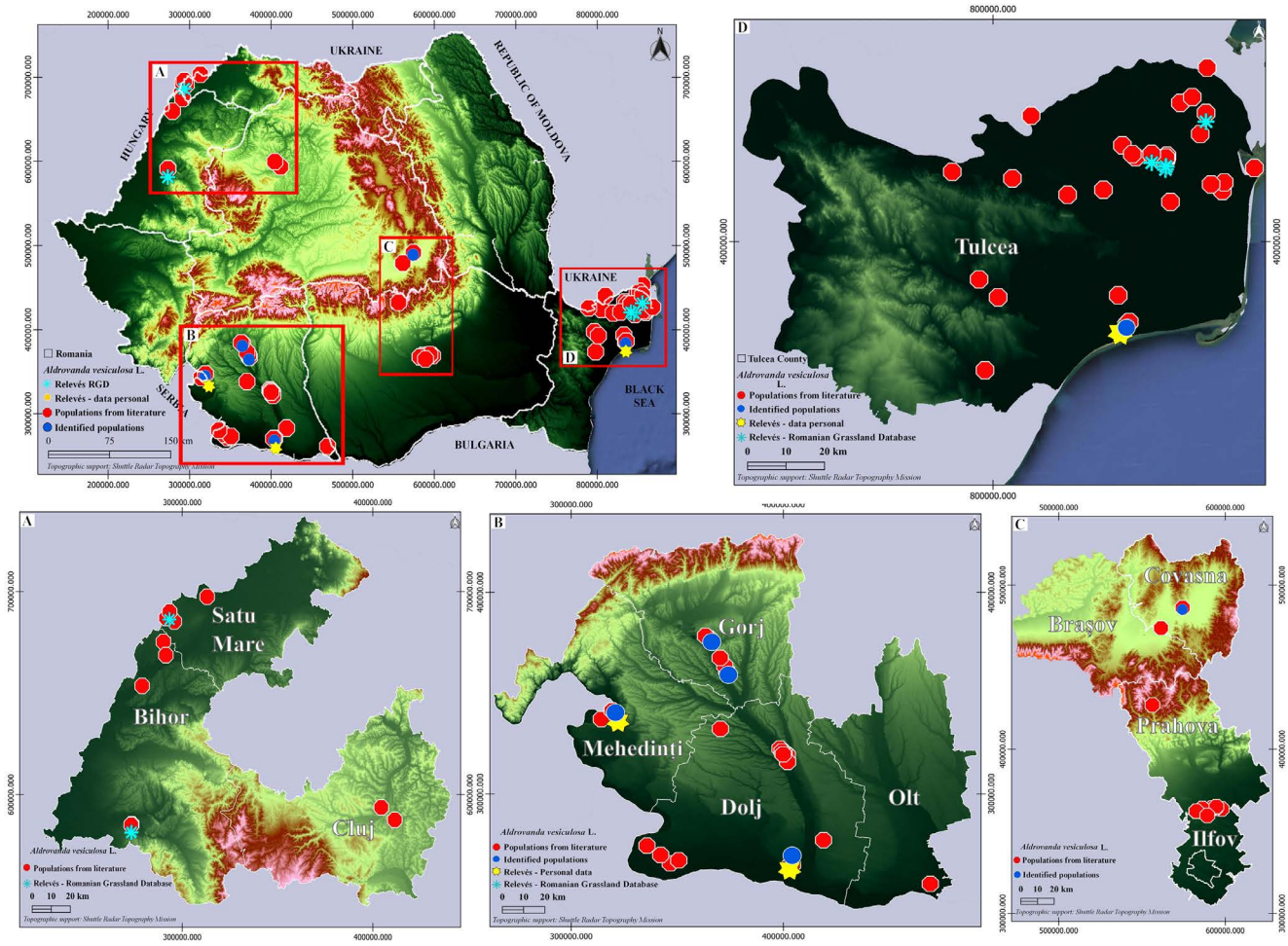

Fig. 3. The distribution of *Aldrovanda vesiculosa* in Romania. (Map: S. D. Chirilă)

adjacent marshes of Chilia Veche (the Bogdaproste area) and C.A. Rosetti (the Magearu Channel; Fig. 2). From Chilia Veche (Bogdaproste area), 30 individuals were relocated on the same day to two areas: Crișan (Ceamurlia polder), hereafter called Crișan (Ceamurlia polder) and Sulina (Vătafu-Împuțita area) in plastic bags, in the same type of plant association (*Typhetum angustifoliae*) and ecological conditions. In the C.A. Rosetti (the Magearu Channel), we relocated 30 individuals to two places (the Letea area and the Răducu Lake area) in each plant association (*Scirpo-Phragmitetum*).

Crișan (Ceamurlia polder; *Typhetum angustifoliae* community). Mesotrophic water with silty soils or fine silty sands with good mineral trophic, slightly clogged areas, and poorly aerated waters. The mean water depth was 30–40 cm; the bottom substrate of the water is rich in organic matter and can easily be lifted into the water as suspensions - pH from 5.9 to 7.7.

Sulina (the Vătafu-Împuțita area; *Typhetum angustifoliae* community). Mesotrophic water with clayey soils, rich in organic matter, and with a slightly acidic (pH = 5.5) or slightly alkaline (pH = 7.5) reaction, the water depth of the channel is between 30–70 cm. The water transparency is 70 cm, and the bottom substrate of the water is rich in organic matter.

As for Letea (the Letea area; *Scirpo-Phragmitetum*), excess moisture tends to suppress the aerobic factors in the soil, and sometimes, slightly salinised soils present substantial accumulations of organic material on the surface. They are interspersed with the clay layer which favours prolonged humidity maintenance during the year. The pH is neutral (7.2), and the mean depth of the channel is 80 cm, similar to the water transparency. The habitat develops at the channel's inbank area, and the water's bottom substrate is rich in organic matter and is about 30 cm thick.

C.A. Rosetti (the Răducu area; *Scirpo-Phragmitetum*). Mesotrophic water with clay or clay-peat soil, temporarily or permanently flooded areas with variable content of mineral salts. It prefers sheltered marshes with shallow water (40–60 cm) with a transparent bottom. The pH is neutral and slightly alkaline. Plant monitoring has been carried out annually since 2017, only once yearly, from July to August. The number and the vitality

of the individuals and the ecological conditions (water transparency, water depth, pH, temperature, substrate, and diagnostic species) were evaluated both in the areas where the species was transplanted and in the areas with the original habitat from where it was collected.

Environmental variables. For each cluster of sites, annual precipitation (BIO12), annual mean temperature (BIO1), and elevation were presented. These values were extracted from the WorldClim database (Fick & Hijmans 2017). Also, the water depth was measured using a topographic site (5 m), and the water temperature was measured with a Hanna HI98494 portable multimeter. The pH was measured with a Portable pH Meter.

RESULTS

Comprehensive update on species distribution: unveiling patterns and trends. In the period 1860–2023, the localities where the species *A. vesiculosa* was reported from Romania were concentrated in the Pontic, Pannonic, Steppic, and Continental bioregions (Fig. 3). A comprehensive analysis of literature records revealed that the species exhibited a pronounced regional distribution pattern, with the highest frequency observed in Dobrogea (22 records, accounting for 33% of the total records). In contrast, the species was less frequent in Oltenia (20 records, 30%), with decreasing frequencies observed in Muntenia (ten records, 15%), Transylvania (eight records, 12%), and Crișana (four records, 6%).

During the period 2021–2023, four previously undocumented populations were confirmed, and two entirely new populations were identified. These newly discovered populations are located in regions where the species is considered to be rare.

Syntaxonomic overview of the nine clusters. *Aldrovanda vesiculosa* was identified in three main habitat types: free-floating aquatic plants, marsh vegetation, and the vegetation of the aquatic plants rooted in the bottom. In this case, the vegetation was classified into three classes, four orders, seven alliances, and 14 plant associations.

Class: *Lemnetea* O. de Bolòs et Masclans 1955
- Order: *Lemnetalia minoris* O. de Bolòs et Masclans 1955
  - All.: *Stratiotion* Den Hartog et Segal 1964
    - Ass.: *Hydrocharidetum morsus-ranae* van Langendonck 1935
    - Ass.: *Stratiotetum aloidis* Nowinski 1930
  - All.: *Lemnion minoris* O. de Bolòs et Masclans 1955
    - Ass.: *Lemno minoris-Spirodeletum polyrhizae* W. Koch 1954
  - All.: *Utricularion vulgaris* Passarge 1964
    - Ass.: *Lemno-Utricularietum vulgaris* Soó (1928) 1947
    - Ass.: *Spirodelo-Aldrovandetum* Borhidi et J. Komlódi 1959

Class: *Phragmito-Magnocaricetea* Klika in Klika et Novák 1941
- Order: *Phragmitetalia* Koch 1926
  - All.: *Phragmition communis* Koch 1926
    - Ass.: *Typhetum angustifoliae* Pign. 1953
    - Ass.: *Schoenoplectetum lacustris* Chouard 1924
    - Ass.: *Scirpo-Phragmitetum* W. Koch 1926
- Order: *Nasturtio-Glycerietalia* Pignatti 1953
  - All.: *Magnocaricion elatae* Koch 1926
    - Ass.: *Eleocharitetum palustris* Savič 1926

Class: *Potamogetonetea* Klika in Klika et Novák 1941
- Order: *Potamogetonetalia* Koch 1926
  - All.: *Nymphaeion albae* Oberd. 1957
    - Ass.: *Myriophyllo verticillati-Nupharetum luteae* W. Koch 1926
    - Ass.: *Nymphaeetum albae* Vollmar 1947
    - Ass.: *Nymphoidetum peltatae* (All. 1922) Bellot 1951
    - Ass.: *Potametum natantis* Hild 1959
  - All.: *Potamogetonion* Libbert 1931
    - Ass.: *Potametum lucentis* Hueck 1931

Aldrovanda vesiculosa is a eurytopic species characteristic of wetland areas. The elevation varied from -1 m to 177 m, with a mean of 12 m. Depending on the study area, the richness of the species ranged from 5 to 26. Perennial plant species predominate (100%) in the analysed communities. The total vegetation coverage ranged from 61% to 100%, with a mean of 82%. The number of relevés was higher in the *Lemnetea* class (35 relevés) compared to the *Phragmito-Magnocaricetea* (21 relevés) and *Potamogetonetea* (15 relevés) classes. The soils are gleyic sandy soils with low to moderate salinity.

The class *Lemnetea* (vegetation of free-floating aquatic plants) corresponds to free-floating duckweed vegetation of the still and relatively nutrient-rich freshwater bodies of the Holarctic region. The number of species ranged between 5 to 25, with 16 species per 25 m^2 and a vegetation cover of 87% (from 61% to 100%). The annual precipitation was in the range of 366 mm to 600 mm, with a mean of 411 mm, and the annual mean temperature ranged from 10°C to 12°C (mean 11.2°C).

The class *Phragmito-Magnocaricetea* (marsh vegetation) corresponds to the reed marsh, sedge beds, and herbland vegetation of the freshwater or brackish water bodies and streams of Eurasia. The richness of species per 25 m^2 was 18 (range 11–26 species), and the vegetation coverage was from 71% to 100%, with a mean of 83%. The annual precipitation varied between 366 mm to 587 mm (mean 379 mm), and the annual mean temperature ranged from 9.8°C to 11.5°C (mean 11.2°C).

The class *Potamogetonetea* (the vegetation of the aquatic plants rooted in the bottom) corresponds to the vegetation of rooted floating or submerged macrophytes

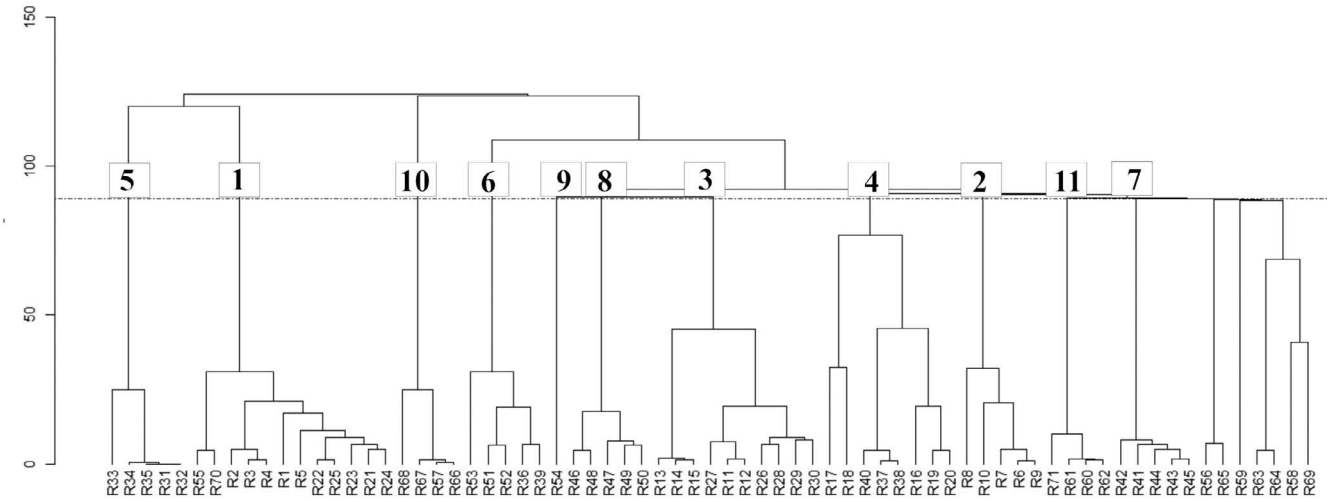

Fig. 4. Dendrogram of relevés with *Aldrovanda vesiculosa* in Romania.

of the stagnant mesotrophic, eutrophic, and brackish freshwater bodies and slowly flowing shallow streams of Eurasia. The species ranged from 6 to 19, with a mean of 10 per 25 m^2. The vegetation coverage varied from 29% to 90% (mean 67%). The annual precipitation ranged from 366 mm to 373 mm (mean 372 mm), and the annual mean temperature ranged from 11.1°C to 11.3°C.

Cluster analysis. The cluster analysis is presented as a dendrogram and synoptic table (Supplementary Table 1). In this context, the vegetation was grouped into 11 clusters (Fig. 4): Cluster 1 includes *Hydrocharidetum morsus-ranae*; Cluster 2 includes *Lemno-Utricularietum vulgaris*; Cluster 3 consists of *Typhetum angustifoliae*; Cluster 4 includes *Lemno minoris-Spirodeletum polyrhizae*; Cluster 5 consists of *Stratiotetum aloids*; Cluster 6 comprises *Spirodelo-Aldrovandetum*; Cluster 7 consists of *Eleocharitetum palustris, Nymphaeetum albae, Potametum lucentis, Potametum natantis,* and *Myriophyllo verticillati-Nupharetum luteae* associations; Cluster 8 includes *Schoenoplectetum lacustris*; Cluster 9 consists of *Scirpo-Phragmitetum*; Cluster 10 includes *Myriophyllo verticillati-Nupharetum luteae*; and Cluster 11 consists of the *Nymphoidetum peltatae* association.

Cluster 1 – The *Hydrocharidetum morsus-ranae* association. Twelve relevés were included in this cluster, distributed in Murighiol (the Perișor area), Crișan (Ceamurlia polder), Letea (the Letea area; Tulcea County), and Gighera (Dolj County). Diagnostic species: *Hydrocharis morsus-ranae* (0.708; 0.001***). The annual mean temperature (11.1°C to 11.6°C) and annual precipitation are moderate (366 mm to 547 mm). The vegetation coverage was 90%, and the number of species varied from 11 to 26 species. The elevation varied from -1 m to 33 m. *Hydrocharis morsus-ranae* L. is a dominant species in this plant association, with a significant presence in all 12 relevés. Its percentages range from 62.5% to 87.5%, indicating consistent and considerable coverage across the entire dataset. The presence of *Aldrovanda vesiculosa* varies from 0.5% to 17.5%. *Lemna minor* and *L. trisulca* are present in most relevés, but with lower percentages than *Aldrovanda vesiculosa*.

Cluster 2 – The *Lemno-Utricularietum vulgaris* association. Five relevés were included and distributed in Murighiol (the Perișor area; Tulcea County) and Ostrovu Corbului (Mehedinți County). Diagnostic species: *Utricularia vulgaris* (0.903; 0.001***) and *Lemna minor* (0.861; 0.001***). The annual mean temperature is 11.7°C (from 11.5°C to 12°C), and the annual mean precipitation is 462 mm (from 375 mm to 591 mm). The vegetation coverage was 61% to 85% (mean 70%). The number of species per 25 m^2 was 12 (from 10 to 15). The elevation ranged from -1 m to 36 m. *Aldrovanda vesiculosa* has low coverage, varying from 0.5% to 5%. The species with a significant presence in these relevés include *Lemna minor* (with percentages from 17.5% to 37.5%) and *Utricularia vulgaris* (from 37.5% to 62.5%). *Hydrocharis morsus-ranae* is present in all the relevés, with relatively constant percentages from 0.5% to 5%. The presence of other species in these environments is comparatively lower, ranging from 0.5% to 5%, signifying a limited diversity within these aquatic habitats.

Cluster 3 – The *Typhetum angustifoliae* association. Within this community, ten relevés were included and distributed in Crișan (Ceamurlia polder) and Murighiol (the Perișor area; Tulcea County). Diagnostic species: *Typha angustifolia* (0.950; 0.001***); *Rorippa amphibia* (0.671; 0.003**); *Phragmites australis* (0.650; 0.013*); *Iris pseudacorus* (0.639; 0.014*); *Symphytum officinalis* (0.632; 0.008**); and *Mentha aquatica* (0.514; 0.022*). The annual mean temperature is 11.3°C (from 11.1°C to 11.5°C), and the annual mean precipitation of 371 mm

(from 366 mm to 376 mm) is moderate. The vegetation coverage is 85% (from 78% to 100%), and the number of species per 25 m^2 was 20 (from 11 to 26 species). Species with a significant presence in these relevés include *Phragmites australis* (5% to 37.5%) and *Typha angustifolia* (with consistent percentages from 62.5% to 87.5%). Also, *Hydrocharis morsus-ranae* is present in most of the relevés, with consistent percentages of 0.5%. The other species have a lower presence in these aquatic environments, varying from 0.5% to 5%, indicating a relatively low diversity. The coverage of *Aldrovanda vesiculosa* ranged from 0.5% to 17.5%.

Cluster 4 – The *Lemno minoris-Spirodeletum polyrhizae* association. Eight relevés were included and distributed in Crișan (Ceamurlia polder; Tulcea County). Diagnostic species: *Ceratophyllum submersum* (0.703; 0.003**); *Ranunculus trichophyllus* (0.652; 0.001***); *Ceratophyllum demersum* (0.566; 0.014*); *Ranunculus aquatilis* (0.561; 0.046*); *Potamogeton nodosus* (0.556; 0.020*); *Azolla filiculoides* (0.507; 0.032*); and *Trapa natans* (0.493; 0.027*). The annual mean temperature was 11.2°C, and the annual mean precipitation was 366 mm. The mean vegetation coverage was 91% (82% to 100%), and the number of species per 25 m^2 was 21 (from 16 to 25). The elevation measured 1 m. *Aldrovanda vesiculosa* is present in all the relevés but with a variation in percentage from 0.5% to 17.5%, indicating a diverse distribution in these relevés. The species with a significant presence in these relevés include *Lemna minor* (with percentages from 17.5% to 62.5%), *Spirodela polyrhiza* (with percentages from 37.5% to 62.5%), and *Hydrocharis morsus-ranae* (with percentages from 5% to 17.5%). Other species, such as *Ceratophyllum demersum, Lemna trisulca, Potamogeton perfoliatus*, and *Typha angustifolia,* are also present in these relevés, but with lower percentages, ranging from 0.5% to 5%.

Cluster 5 – The *Stratiotetum aloidis* association. In this cluster, five relevés were included and distributed in Crișan (Ceamurlia polder; Tulcea County). Diagnostic species: *Stratiotes aloides* (0.922; 0.001***), *Oenanthe aquatica* (0.523; 0.007**), and *Stuckenia pectinata* (0.463; 0.017*). The annual mean temperatures were moderate (11.19°C), and the annual mean precipitation was low (366 mm). The mean vegetation cover was 93% (from 73% to 98%), and the number of species per 25 m^2 was 12 (from 12 to 13). The elevation was 1 m. The species *Aldrovanda vesiculosa* has a consistent presence of 0.5% in each relevé, indicating a uniform distribution in these environments. The species *Hydrocharis morsus-ranae* and *Stratiotes aloides* are present in all the relevés, with variable percentages. The dominant species in these relevés is *Stratiotes aloides*, with a significant presence of 87.5% in most of the relevés. *Hydrocharis morsus-ranae* has a consistent presence, varying from 5% to 17.5%. The negligible presence (0.5% or absent) of other species in these relevés indicates the low species diversity of these aquatic environments.

Cluster 6 – The *Spirodelo-Aldrovandetum* association. This cluster includes five relevés distributed in Crișan (Ceamurlia polder; Tulcea County) and Cărăsău (Bihor County). Diagnostic species: *Spirodela polyrhiza* (0.887; 0.001***); *Aldrovanda vesiculosa* (0.847; 0.001***); *Lemna gibba* (0.723; 0.004**); and *Hottonia palustris* (0.588; 0.013*). The annual mean precipitation was 506 mm (from 366 mm to 600 mm), and the annual mean temperature was 10.5°C (from 10°C to 11.1°C). The vegetation coverage varied from 71% to 95% (mean 84%), and the number of species per 25 m^2 was 10 (from 5 to 16). The difference in elevation was in the range of 1 m to 177 m. The species *Aldrovanda vesiculosa* has a coverage from 37.5% to 62.5%. Other species, such as *Ceratophyllum demersum*, *Lemna gibba, L. minor, Najas minor, Phragmites australis, Sagittaria sagittifolia,* and *Spirodela polyrhiza*, are also present, with relatively consistent percentages from 0.5% to 37.5%. Species such as *Salvinia natans* and *Typha angustifolia*, were also present in some of the relevés with percentages of 5%. On the other hand, *Spirodela polyrhiza* and *Typha angustifolia* have a significant presence in several relevés, suggesting adaptation to a wider variety of aquatic environments.

Cluster 7 – The *Eleocharitetum palustris, Nymphaeetum albae, Potametum lucentis, Potametum natantis,* and *Myriophyllo verticillate-Nupharetum luteae* associations. This cluster includes five relevés distributed in Crișan (Ceamurlia polder; Tulcea County). Diagnostic species: *Potamogeton natans* (0.713; 0.017*); *Nymphaea alba* (0.643; 0.031*); and *Potamogeton pusillus* (0.535; 0.041*). Associations were identified for *Eleocharitetum palustris*: *Eleocharis palustris* (0.998; 0.001***); *Equisetum palustre* (0.775; 0.001***); *Lycopus europaeus* (0.700; 0.001***); *Carex riparia* (0.692; 0.004**); *Phalaroides arundinacea* (0.645; 0.018*); *Stachys palustris* (0.632; 0.026*); *Carex acutiformis* (0.600; 0.005**); *Scutellaria galericulata* (0.572; 0.009**); *Galium palustre* (0.560; 0.008**); and *Veronica beccabunga* (0.516; 0.049*). The number of species was 6 to 17, with 12 per 25 m^2. The vegetation coverage varied from 29% to 78% (mean 66%). The annual mean temperature was from 11.12°C to 11.19°C (mean 11.15°C), and the annual mean precipitation varied from 366 mm to 373 mm (mean 370 mm). The elevation was 1 m. The dominant species are *Eleocharis palustris*, *Nymphaea alba*, *Nuphar lutea*, *Potamogeton lucens,* and *Potamogeton natans*.

Cluster 8 – The *Schoenoplectetum lacustris* association. This cluster includes five relevés distributed in Crișan (Ceamurlia polder; Tulcea County). Diagnostic species: *Schoenoplectus lacustris* (0.987; 0.001***); *Sparganium erectum* (0.924; 0.001***); *Lysimachia vulgaris* (0.766; 0.001***); *Lythrum salicaria* (0.717; 0.001***); *Ranunculus lingua* (0.702; 0.002**); *Rumex hydrolapathum* (0.678; 0.003**); *Alisma plantago-aquatica* (0.535; 0.007**); and *Butomus umbellatus* (0.509; 0.003**). The number of species per 25 m^2 was 18 (from 16 to 21 spe-

Table 1. The characteristics of sites with *Aldrovanda vesiculosa* in Romania.

Locality	County	Latitutude, longitude	Habitats	Plant association	Number of individuals	Area (m^2)	pH	Water depth	Population status
Crișan (Ceamurlia polder)	Tulcea	45.212940°, 29.438760°	fish farm	*Scirpo-Phragmitetum, Hydrocharidetum morsus-ranae, Typhetum angustifoliae, Lemno minoris-Spirodeletum polyrhizae, Stratiotetum aloidis, Spirodelo-Aldrovandetum, Eleocharitetum palustris, Nymphaeetum albae, Potametum lucentis, Potametum natantis, Myriophyllo verticillate-Nupharetum luteae, Schoenoplectetum lacustris*	500–600	50	5.9–7.7	30–80 cm Water transparency 80 cm	present
Sulina (Vătafu-Împuțita area)	Tulcea	45.130710°, 29.524690°	marsh	*Scirpo-Phragmitetum, Typhetum angustifoliae*	30–40	50	5.5-7.5	30–70 cm Water transparency 70 cm	extinct
Letea (Letea area)	Tulcea	45.182480° 29.280575°	marsh	*Scirpo-Phragmitetum, Hydrocharidetum morsus-ranae*	30–40	50	7.2	60–90 Water transparency 90 cm	extinct
C.A. Rosetti (Răducu area)	Tulcea	45.234736°, 29.462014°	shallow lake	*Scirpo-Phragmitetum*	30–40	50	7.1	40–60 cm Water transparency 60 cm	extinct
Murighiol (Perișor area)	Tulcea	44.809743°, 29.239652°	fish farm	*Hydrocharidetum morsus-ranae, Lemno-Utricularietum vulgaris, Typhetum angustifoliae*	500–1000	50	7.4	10–50 cm Water transparency 50 cm	present
Chilia Veche (Bogdaproste area)	Tulcea	45.130466°, 29.213682°	marsh	*Typhetum angustifoliae*	100–200	50	6.8	30–50 cm Water transparency 50 cm	present
Gighera	Dolj	43.842489°, 23.808104°	marsh	*Hydrocharidetum morsus-ranae*	200–300	50	5.9	10–30 cm Water transparency 30 cm	present
Ostrovu Corbului	Mehedinți	44.515113°, 22.724652°	marsh	*Lemno-Utricularietum vulgaris*	200–400	50	7.3	10–50 cm Water transparency 50 cm	present
Cărăsău	Bihor	46.695861°, 22.036029°	water storage	*Spirodelo-Aldrovandetum*	200–300	50	6.5	30–50 cm Water transparency 50 cm	extinct
Sanislău	Satu Mare	47.643217°, 22.247358°	marsh	*Scirpo-Phragmitetum*	200–300	50	7.1	10–50 cm Water transparency 50 cm	extinct
Ceplea	Gorj	44.704158°, 23.397886°	marsh	*Scirpo-Phragmitetum*	200–400	50	6.8	10–30 cm Water transparency 30 cm	present
Peșteana-Jiu	Gorj	44.846912°, 23.291188°	marsh	*Scirpo-Phragmitetum*	100–300	50	6.7	10–30 cm Water transparency 30 cm	present
Reci	Covasna	45.823079°, 25.928827°	marsh	*Spirodelo–Aldrovandetum*	50–100	50	6.9	30–50 cm Water transparency 50 cm	present

cies), and the mean vegetation cover was 87% (from 77% to 97%). The annual mean precipitation was 366 mm (from 366 mm to 367 mm), and the annual mean temperature was 11.19°C (11.19°C to 11.22°C). The elevation was 1 m. *Aldrovanda vesiculosa* maintains a constant presence in all five relevés, with a distribution of 0.5% in each of them. *Schoenoplectus lacustris* remains dominant, with a continuous presence of 62.5% in all five relevés.

Cluster 9 – The *Scirpo-Phragmitetum* association. This cluster includes one relevé distributed in Sanislău (Satu Mare County). There were 20 species, and the vegetation cover was 94%. The annual mean temperature was 9.8°C, and the annual mean precipitation was 587 mm. The elevation was 140 m. *Aldrovanda vesiculosa* had a coverage of 0.5%. The dominant species was *Phragmites australis*, with a coverage of 62.5%.

Cluster 10 – The *Myriophyllo verticillati-Nupharetum luteae* association. Four relevés, distributed in Crișan (Ceamurlia polder; Tulcea County), were included in this cluster. Diagnostic species: *Nuphar lutea* (0.998; 0.001***); and *Potamogeton crispus* (0.490; 0.014*). The vegetation coverage was from 67% to 90% (mean 73%), and the number of species per 25 m^2 was 9 (from 6 to 11 species). The annual mean temperature was 11.1°C, and the annual mean precipitation was 372 mm. The elevation was 1 m. Species such as *Nuphar lutea* and *Nymphaea alba* have a significant and constant presence in all four relevés, with *Nuphar lutea,* in particular, having a higher coverage, increasing up to 87.5%. Most of the other species show a relatively constant presence or are absent in some relevés, such as *Stratiotes aloides* and *Utricularia vulgaris,* which are present only in certain relevés but in small proportions. *Aldrovanda vesiculosa* had a coverage of 0.5% in all four relevés.

Cluster 11 – The *Nymphoidetum peltatae* association. Four relevés were included and distributed in Crișan (Ceamurlia polder) and Letea (the Letea area; Tulcea County). Diagnostic species: *Nymphoides peltata* (0.994; 0.001***); and *Hippuris vulgaris* (0.729; 0.004**). The vegetation coverage was 73% (from 67% to 90%), and the number of species per 25 m^2 was 12 (from 9 to 19 species). The elevation was 1 m. These communities were recorded in areas with an annual mean precipitation of 373 mm (from 372 mm to 373 mm) and annual mean temperatures of 11.17°C (from 11.12°C to 11.32°C). Some species exhibited a consistent presence in all four relevés, such as *Nymphoides peltata*, with a constant coverage of 62.5%. Other species, such as *Aldrovanda vesiculosa*, *Butomus umbellatus,* or *Ceratophyllum demersum*, are present in small proportions, with values of 0.5%, or absent in certain relevés. *Hippuris vulgaris* and *Salvinia natans* significantly vary in their coverage in the four relevés, from 0.5% to 5%. Species such as *Utricularia vulgaris* or *Stratiotes aloides* are present in small proportions and are absent in some relevés.

Species translocation. In the Crișan area, the species constantly developed (dozens of individuals) for up to six years. They overcame the destruction of their habitat caused by covering the channels with soil deposits as part of hydro-technical works. In Sulina (the Vătafu-Împuțita area), the species survived in the first three years with a gradual reduction of the overwintering individuals until they became extinct, but without any changes in the habitat being observed. In the areas of Letea and Răducu, the species was relocated from "ex-situ" and survived in the natural environment only in the first two years. In both sites, the water level (20–90 cm) dropped to 5–10 cm or completely dried up in some places.

DISCUSSIONS

Species distribution. The distribution of the species *Aldrovanda vesiculosa* in Romania appears to be narrower than previously reported in the literature (Oprea 2005; Kaminski 2006; Dihoru & Negrean 2009). In the past, *Aldrovanda vesiculosa* was identified in 66 localities in Romania. Currently, the species has been registered in six localities: Reci (Covasna County); Gighera (Dolj County); Pește ana-Jiu and Ceplea (Gorj County); Ostrovu Corbului (Mehedinți County); and Murighiol (the Perișor area; Tulcea County). Recently, the species was identified in three other localities in Gorj County (Bâlteni, Izvoarele, and Sărdănești; Răduțoiu *et al.* 2023). The main reasons for the disappearance of the species in Romania are the eutrophication of agricultural sites, fishing, pollution, and the lowering of the water level. Thus, Dihoru & Negrean (2009) suggested that various anthropogenic activities, such as the drying and pollution of ponds, have destroyed the populations in Romania. Furthermore, the discharge of different toxic substances has killed the tiny organisms (*Cyclops* sp., *Daphnia* sp., *Cypris* sp., etc.) which the plant digests (Beldie *et al.* 1955). Our results indicate that 89% (66 records from the literature) of the reports from the literature with *A. vesiculosa* have not been confirmed in the last two decades. Six new locations of *A. vesiculosa* have been identified in the Oltenia region in the previous two years. In this context, the species is considered critically endangered nationally (Dihoru & Negrean 2009). The severe changes in environmental conditions explain this phenomenon. According to the EEA (https://eunis.eea.europa.eu/species/168992; EEA 2023), 23 Natura 2000 sites have been designated to protect the waterwheel. Of these, two sites are located in Bulgaria (9%), two sites in Lithuania (9%), three sites in Hungary (13%), six sites in Romania (26%) and ten sites are in Poland (43%). The species was reported in another Natura 2000 site in Romania - ROSPA0011 Blahnița during the study period.

In the Transylvania region, the species was recorded in four sites in the past (Oprea 2005; Dihoru & Negrean 2009; Petráss *et al.* 2010), of which only one

site was confirmed (Mestecănișul de la Reci - Covasna County). The populations we identified range from 50 to 100 individuals in an area of 50 m^2 (Table 1).

In the Oltenia region, the species was recorded in 24 sites (Oprea 2005; Dihoru & Negrean 2009), of which seven sites were recently registered (Gighera - Dolj County; Ostrovu Corbului - Mehedinți County; Ceplea, Izvoarele, Sărdăneşti, Bâlteni, and Peşteana-Jiu - Gorj County). The populations we identified range from 100 to 400 individuals in an area of 50 m^2. The main factors negatively affecting the species in this region are the decrease in the water level and eutrophication. The species was identified in marshes at 10 cm to 50 cm, on the water surface, or near it (Table 1).

In the Dobrogea region, the species was recorded in 22 sites in the past (Oprea 2005; Dihoru & Negrean 2009). Currently, the species is only found in Murighiol (the Perișor area) and Crișan (Ceamurlia polder). Although the species is abundant and concentrated in these sites, it is subject to pressures due to climate variability or land use. *A. vesiculosa* was detected inhabiting the channels within fish and agricultural polders, where occurrences of natural vegetation were observed. In Murighiol (the Perișor area), in an abandoned fish farm built up as a network of canals, the population was estimated as comprising from 500 to 1000 individuals, on 50 m^2. The species was identified from 10 cm to 50 cm depth at 16°C to 17.8°C, constantly measured by a datalogger for a month (July). In Crișan (Ceamurlia polder), the population consists of 500 to 600 individuals, on 50 m^2 (Table 1).

The waterwheel has not been identified in the other regions of Romania (Crișana, Maramureș, and Muntenia).

Eco-coenotic analysis. *Aldrovanda vesiculosa* grows in artificial habitats in Romania, namely fish farms. In these habitats, the species prefers littoral areas of lakes and canals characterised by low-height palustrine vegetation. It tends to thrive in locations with sparsely distributed aquatic vegetation shaded by cattails and reed beds. It is important to note that this species is protected in many countries and may be sensitive to changes in water quality and its habitat. Studies and careful monitoring of *A. vesiculosa* populations are essential for conserving this unique and interesting plant. According to the data collected from the field, the study results showed that this species prefers the habitat-type vegetation of free-floating aquatic plants (*Lemnetea*). In the marsh habitats (*Phragmito-Magnocaricetea*) and vegetation of aquatic plants rooted in the bottom (*Potamogetonetea*), the presence of the species was low.

According to the literature (Convention on the Conservation of European Wildlife and Natural Habitats 2019; Chytry *et al.* 2020), *A. vesiculosa* corresponds to the following types of EUNIS habitats: (1) wetlands - Helophyte beds (Q5) - Tall-helophyte beds (Q51), Tall-sedge beds (Q53); (2) inland surface waters - Surface standing waters (C1) - Permanent eutrophic lakes, ponds and pools (C1.3) - Free-floating vegetation of eutrophic waterbodies (C1.32).

The most common plant species identified in the relevés with *Aldrovanda vesiculosa* were *Ceratophyllum demersum, Lemna minor, Phragmites australis, Salvinia natans, Hydrocharis morsus-ranae* and *Typha angustifolia*. In the case of plant associations, the species frequently occurs in the associations *Hydrocharidetum morsus-ranae* (12 relevés - 17%), *Lemno minoris-Spirodeletum polyrhizae* (8 relevés – 11%), and *Typhetum angustifoliae* (7 relevés - 10%). The most frequent alliances are *Stratiotion* (17 relevés - 24%) and *Phragmition communis* (16 relevés - 23%). Most relevés are included in the *Lemnetalia minoris* order (35 relevés - 49%).

The associations in which *A. vesiculosa* has been mainly threatened are - *Lemno-Utricularietum vulgaris* - Ostrovu Corbului, *Scirpo-Phragmitetum*, *Typhetum angustifoliae* and *Hydrocharidetum morsus-ranae* – Crișan (Ceamurlia polder).

CONCLUSIONS

The distribution analysis of the species *A. vesiculosa* indicates a narrowing of the area in Romania. Given this species' stenobiont nature, conservation managers have traditionally perceived these habitats as relatively inconspicuous. Nevertheless, it is essential to underscore that any alteration in the factors limiting their existence may be pivotal in determining the sustainability of these populations. The primary existential threat to *A. vesiculosa* lies in the gradual loss of its habitat due to drying up, which results from factors such as animal trampling (grazing), substantial modifications to land use (direct and radical changes), or alterations in the surrounding environs. From a phytocoenological point of view, the species prefers communities of *Hydrocharis morsus-ranae* and *Typha angustifolia*. The species' ecological requirements include high water transparency, minimal or no eutrophication, clean, shallow waters, and a source of water originating from infiltration. Continuous monitoring of the species' habitat and potential relocation sites over a minimum of five years, followed by selecting the most favourable habitat conditions for *A. vesiculosa* to thrive, may significantly enhance the success of translocation efforts. Adequate and stable hydrological conditions should be considered a fundamental prerequisite for identifying potentially suitable *Aldrovanda* sites.

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REZIME

Botanica SERBICA

Fitocenologija, distribucija i preferencije staništa vrste *Aldrovanda vesiculosa* (Droseraceae) u Rumuniji

Simona Dumitrița Chirilă, Mihai Doroftei, Kiril Vassilev i Silviu Covaliov

Aldrovanda vesiculosa je ugrožena akvatična vrsta u Rumuniji koja raste u plitkim vodama gde je kompeticija sa drugim vrstama slaba ili izostaje. Trenutno nedostaje sveobuhvatan pregled distribucije vrste i preferencija staništa u Rumuniji, koje je ova studija stoga imala za cilj da ažurira. Istraživanja su vršena na lokalitetima na području Obrudže, Oltenije, Muntenije, Transilvanije i Krisane. U cilju vegetacijske klasifikacije analiziran je 71 snimak sa *A. vesiculosa*. Veličina uzorkovanih površina je bila 4 m^2 to 25 m^2. U cilju sintaksonomskog grupisanja korišćen je aglomerativno hijerarhijsko klasterovanje. Optimalan broj klastera je izabran korišćenjem korigovanih indeksa Rand i Silhouette. Dijagnostičke vrste su identifikovane za svaki klaster na osnovu indeksa vrednosti indikatora (IndVal). Rezultati studije su pokazali da dominantna većina prethodnih populacija *A. vesiculosa* (89%) više nije identifikovana iz Rumunije u periodu 2008–2021. Nedavno (2021–2023) pronađeni su samo na šest lokaliteta u oblastima Dobrudže, Transilvanije i Oltenije. Uzroci nestanka vrste su eutrofikacija i snižavanje vodostaja. Sa fitocenološke tačke gledišta, identifikovano je 11 biljnih zajednica u kojima se nalazi *A. vesiculosa*. Prema klasifikaciji EUNIS, ova vrsta naseljava močvarna područja (K) i unutrašnje površinske vode (C). U zaključku, translokacija se predlaže kao primarna mera očuvanja za zaštitu vrste.

Ključne reči: karakteristike staništa, fitocenologija, novija rasprostranjenost, translokacija, vodeni točak.