Biological flora of Central Europe: *Aldrovanda vesiculosa* L

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A B S T R A C T

Aldrovanda vesiculosa L. (Droseraceae) is a very rare and critically endangered submerged aquatic carnivorous plant with snapping traps which grows in shallow, standing dystrophic waters such as: lakes, dam reservoirs, peaty fishponds, pools in peat bogs and fens, backwater pools and oxbows in floodplains and basins of large rivers. While it covers a vast territory of four continents in the Old World across various climatic zones, its recent natural fragmentary spread includes only around 50 sites worldwide following a marked population decline during the last 150 years. The major extant world sites occur in Ukraine, Poland, Romania and W Russia. The plant exhibits a marked physiological polarity along its linear shoot with rapid apical shoot growth. Flowering and especially seed-set are rare and stimulated by high temperatures, yet the plants propagate mainly vegetatively by branching. Seeds probably form a seed bank and colonise new sites by being transferred by water birds. All temperate and some (sub)tropical populations form winter buds (turions), which sink to the bottom in autumn and actively rise to the water surface in spring. The world population of *Aldrovanda* is genetically rather uniform which may be caused by at least one recent bottleneck followed by long-distance dispersal by water birds combined with the founder effect, low mutation rates and dominant asexual reproduction. *Aldrovanda* is stenotopic and although it is relatively tolerant of many individual ecological factors, it requires an optimal combination of many ecological habitat factors and even small changes in this combination can lead to its decline or extinction. The most important ecological requirements are: high CO_2 concentration in the water (> 0.1 mM), shallow water of at least 0.1 m, habitats free of dense stands of either submerged, free-floating or emergent plants or filamentous algae, a thick layer of slowly decomposable plant litter on the bottom and abundant zooplankton as prey. The main threats to its sites are water eutrophication and water level decline. The introduction of *Aldrovanda* to potentially suitable sites has become an efficient way to conserve its endangered world population in Switzerland, Poland, Czech Republic, The Netherlands, Australia and Japan.

1. Introduction

Aldrovanda vesiculosa L. (Droseraceae; English: Waterwheel plant; German: Wasserfalle; French: aldrovandie à vessies, aldrovandie vésiculeuse; Italian and Hungarian: aldrovanda) is a very rare and critically endangered aquatic carnivorous plant with snapping traps exhibiting spectacular, rapid movement. It is rootless, free-floating just below the water surface in shallow, standing dystrophic (humic) waters (Fig. 1; Lloyd, 1942; Berta, 1961; Huber, 1961; Adamec, 1995; Weber, 1995; Cross, 2012; Fleischmann et al., 2018a). Although it covers a vast territory including all continents of the Old World and across various climatic zones from NE Russia (ca. 60.5 °N) to SW Australia (ca. 34 °S), recent work determining its natural spread indicated only around 50 sites worldwide. Cross (2012) confirmed that it is extremely scarce and irregular in the time and area and has vanished from many countries and regions during the last 50 years. *Aldrovanda vesiculosa* (AV) has always attracted the curiosity of ecologists and physiologists due to its

remarkable rapid trap movement and carnivorous habit. Moreover, in the last about 40 years, it also has attracted the attention of nature conservationists due to its rapid decline in many European sites and in Japan (e.g., Walters, 1979; Adamec, 1995, 2005a; Weber, 1995; Adamec and Lev, 1999; Cross, 2012).

Numerous pieces of knowledge on the biology of AV were comprehensively reviewed by Lloyd (1942) in his monograph on carnivorous plants. Berta (1961) summarised the scattered ecological field data on AV as well as its world distribution. Degreef (1986) in his French review summarised the biological data on AV with an emphasis on the older literature from the 19th century. A couple of studies by Kamiński (1987a, 1987b) on natural Polish AV sites represented a breakthrough in the modern study of field- and laboratory-based ecophysiology of AV and has led consequently to the specification of remaining European AV sites and ecological requirements of this stenotopic species (Adamec, 1995). Invaluable information on AV biology has been obtained after the reliable outdoor culture of AV was

Fig. 1. Branched shoots of AV (from E Poland) at an artificial site in a fen lake Karštejn, S Bohemia, Czech Republic.

established at the Institute of Botany at Třeboň, Czech Republic, and the Botanical Garden of the University of Wroclaw, Poland (published in Adamec, 1997a; Kamiński, 2006). In this way, hundreds of plants from a Polish AV population have become available for various field- and laboratory-based experiments, which both significantly enhanced the ecophysiological knowledge of AV and its ecological requirements and also markedly contributed to the introduction of AV to potentially suitable sites in Central Europe as an effective measure to conserve its declining European population (Adamec, 1997b, 1999a, 1999b, 2000, 2003, 2005a, 2008a, 2008b, 2016; Adamec and Lev, 1999; Adamec and Kovářová, 2006; Kamiński, 2006; Adamec et al., 2010; Adamec and Kučerová, 2013a, 2013b; Poppinga et al., 2018; Westermeier et al., 2018). The increasing volume of biological knowledge on AV and the urgent need to accept measures for its worldwide conservation stimulated the preparation of a monograph on AV biology (Cross, 2012). All pieces of knowledge on the ecophysiology of AV have been reviewed thoroughly by Adamec (2011a, 2018a), while Poppinga et al. (2018) have reviewed the biophysical aspects of the trap functioning. The aim of the present paper is to review all biological properties of AV with an emphasis on temperate European populations.

2. Morphology and taxonomy

2.1. Vegetative morphology and growth traits

Aldrovanda vesiculosa is a perennial, rootless, free-floating submerged carnivorous plant with a linear shoot 6–20 (rarely 30–40) cm long (Fig. 1). The poorly branched, thin stem with a highly modular structure bears regular successive leaf whorls of 6–9 (mostly 7 or 8) leaves terminating into a carnivorous snapping trap with bristles. The trap consists of a two-lobed lamina (2–6 mm long) with a midrib and bears 3–6 long bristles (3–7 mm long) at its apical end (Lloyd, 1942; Kamiński, 1987a; Cross, 2012). Adult plants bear a shoot apex and usually 15–20 mature leaf whorls. Regularly arranged leaves with traps within each whorl (leaf node) are reminiscent of a water wheel, giving the plant its English name. The total length of the leaves with bristles is 7–15 mm. The shoots exhibit a marked and very steep structural, growth and physiological polarity (Adamec, 2000, 2011a, 2018a; Cross, 2012; Šimura et al., 2016). The principal feature of this polarity is the rapid apical shoot growth of 1–1.5 new leaf whorls per day, while the basal shoot part is continuously senescing and decaying so that the total shoot length is approximately constant (the 'conveyer-belt' system of shoot growth; Adamec, 2000, 2008a, 2016; Adamec and Kovářová, 2006; Adamec et al., 2010). Other features of the shoot polarity are related to tissue mineral nutrient content and nutrient reutilization, allocation of prey-derived nutrients, carbohydrate content, photosynthetic and respiration rates and phytohormonal content (for a

review see Adamec, 2011a, 2018a). Like the morphologically and ecologically similar, but not closely related, aquatic carnivorous plants of the genus *Utricularia* (Lentibulariaceae), rapid apical shoot growth and turnover are necessary to counterbalance shoot covering by epiphytic and filamentous algae and to allow rapid vegetative propagation over the relatively short growing season in temperate climates (Adamec, 1999a).

Although temperate AV populations can flower prolifically under favourable conditions and even set fertile seed, it is generally observed that they propagate predominantly vegetatively by apical branching of the shoots (Kamiński, 1987a; Adamec, 1995, 1999a; Adamec and Lev, 1999; Cross, 2012; Cross et al., 2015, 2016). Under favourable growth conditions, branches based in shoot apices are formed fairly regularly and on average after each successive 5–7 (range 2–11) leaf whorls ('branching rate'). Adult plants may bear 1–8 branches, usually only 2–4, but a great deal of adult plants can also be non-branched. When expressed as the time needed for the formation of the next branch, the 'branching frequency' (branching rate divided by apical shoot growth rate) was ca. 5 d in a field-growth experiment (Adamec and Kovářová, 2006). Therefore, a high branching frequency indicates optimal ecological growth conditions and is also the crucial prerequisite for a high relative growth rate (RGR) as all new plant biomass (except for flowering) is allocated only to branches (Kamiński, 1987a; Adamec, 1999a, 2000; Adamec and Kovářová, 2006). During a seasonal field-growth experiment involving Polish plants, older attached branches started forming visible secondary branches only after on average 13.6 mature leaf whorls (range 8–19), and at about 11–14 whorls when separated. A distinct competition exists between the apical growth rate of main shoots and young branches: the growth rate of young branches (< 6 mature whorls) is only about 67% of that of the main shoot, but is the same in older branches (Adamec, 1999a). All branches separate sooner or later from the mother shoot giving a rise to a new plant or turion (winter bud). For a non-destructive growth analysis or a population size assessment, it is thus very convenient and easy to count all visible branches in all plants within the sample and use these counts of all apices for calculations instead of biomass data (Adamec, 1999a; Adamec and Lev, 1999; cf. Adamec and Kovářová, 2006; Adamec et al., 2010).

As a result of high branching frequency and apical shoot growth rate in AV plants under favourable summer conditions, the total growth rate of individual plants or a micropopulation is also very high. Both in terms of the total number of apices in shoots and branches or the total shoot biomass, the mean doubling time of biomass (i.e., 0.693/RGR) in the field or culture is just 8.5–28.7 days for Polish plants (Adamec, 1999a, 2000, 2011a, 2018a; Adamec and Lev, 1999; Adamec and Kovářová, 2006; Adamec et al., 2010) and similarly 7.4–24.7 days in the field for subtropical plants in SW Australia (Cross, 2012). High RGR correlates with high apical shoot growth rate. These values confirm the very high growth rate of AV under favourable summer growth conditions and suggest that AV follows the R-strategy (*sensu* Grime, 1979). In a seasonal field-growth experiment in the Czech Republic, one short non-branched plant gave an exponential rise to 8–35 shoot apices over a 3 month growth period (Adamec, 1999a). Nevertheless, in spite of the potential rapid exponential growth, AV stands only rarely attain very high vegetation coverage > 60-80% and/or > ca. 600 adult plants/m^2 at the peak of the growing season (cf. Kamiński, 1987a; Adamec, 1999a, 2005a; Adamec and Lev, 1999; Cross et al., 2015, 2016). The reasons for this may arise from competition with other plant species (or self-competition) or filamentous algae (shading), a shortage of dissolved free CO_2 concentration or mineral nutrients, low prey availability in the ambient water, or a large proportion of the AV population during autumnal turion formation or spring turion sprouting being grazed by water birds or dying over the winter (Adamec, 1999a; Adamec and Lev, 1999).

The partly translucent snapping traps of foliar origin are 2–6 mm large (Fig. 1; Lloyd, 1942; Juniper et al., 1989; Cross, 2012; Poppinga

et al., 2018). The AV trap is attached below the bristles to the petiole and consists of two semicircular lobes connected by a continuation of the petiole midrib. The petiole usually contains large, distinct air lacunae which contribute to plant flotation and give the plant a light coloration. Traps (especially when closed) are usually twisted to one side and also partly backwards. In fully open traps, the angle between two convex lobes is ca. 90°. The inner part of a trap near to the midrib consists of three cell layers, while the outer part consists of two. Recent knowledge of the mechanism of trap closing has been thoroughly reviewed by Poppinga et al. (2018). After mechanical irritation in warm water, the trap can close within only 10 ms. The rapid closure of AV traps is caused mainly by rapid turgor changes in the 'motor zone' of the trap near the midrib, but a release of a stored elastic energy of the bent midrib is also considered (Westermeier et al., 2018).

Specific types of glandular hairs occur prolifically on the inner trap surface (Lloyd, 1942; Juniper et al., 1989; Cross, 2012; Poppinga et al., 2018): ca. 30–40 long, thin trigger hairs, many mushroom-shaped, stalked tetrameric digestive glands and quadrifid (X-shaped) glands. In the traps, the secretion of digestive hydrolytic enzymes is not constitutive, but is stimulated by trap closure (Muravnik et al., 1995; Płachno and Muravnik, 2018). AV effectively captures small aquatic animals with a body size of usually ca. 0.6 to 3 mm but usually only a small proportion of traps has any prey item (Akeret, 1993; Cross, 2012; Darnowski et al., 2018). Horstmann et al. (unpubl. res.) have recently studied the spectrum of prey captured at one artificial German and four artificial Czech AV sites: the most common prey taxa were Crustacea, Ostracoda, Arachnida (Hydrachnidia), fine larvae of Nematocera (i.e., Diptera, mosquito larvae) and Ephemeroptera and fine Mollusca (snails). Akeret (1993) also reported a similar prey spectrum from a Swiss site. These results suggest that AV traps do not possess any distinct prey selectivity and capture opportunistic prey which is available. Prey capture always leads to a marked growth enhancement: increases in apical shoot growth rate and branching are observed (Kamiński, 1987a; Adamec, 2000, 2008a, 2011a, 2018a; Adamec et al., 2010).

2.2. Taxonomy and evolution

Aldrovanda L. is a monotypic genus containing only a single extant species *A. vesiculosa* L. It is placed in the family Droseraceae and the order Nepenthales (or 'non-core Caryophyllales'*sensu* APG III; Cross, 2012; Fleischmann et al., 2018a,b). Its generic name honours the founder of the Botanic Garden in Bologna, Italy, Ulisse Aldrovandi, while its species name is based on the Latin *vesicula* (small bladder). Although this species is naturally distributed over a huge territory on four continents of the Old World across several climatic zones and consists of temperate and (sub)tropical populations, it is considered taxonomically united, without subspecies (Cross, 2012; Fleischmann et al., 2018a). Old historical synonyms and descriptions of several varieties are not considered taxonomically recently (see Cross, 2012). On the basis of the production of anthocyanins leading to red or reddish coloration of the shoots, a new variety, *A. vesiculosa* var. *rubescens* A.Cross and L.Adamec, has recently been described (Cross, 2012). The red coloration in AV is a highly stable and inherited trait and is based on several genes (Elansary et al., 2010; Cross, 2012). This variety comprises the following world populations which can be red or reddish under favourable growth conditions: all (sub)tropical Australian, East Timor and African populations, the only Hungarian population from Baláta-tó (Lake Baláta; Cross, 2012) and a newly discovered population from NE China (Y. Yunlong, pers. observ.) However, it is not known whether the extinct Indian and subtropical Asian AV populations also had such a red coloration. Thus, all other temperate AV populations do not produce anthocyanins and are only green. Nevertheless, a closer taxonomic relatedness between the red temperate Hungarian population and the red (sub)tropical populations from the southern hemisphere has not been proven (Elansary et al., 2010; Cross, 2012). The former authors consider all green temperate AV populations as loss

mutants for anthocyanin production, whereas the red Hungarian one as a reversion mutant. For the above reasons, Thiele and Coffey (2014) regarded the var. *rubescens* as a mere colour morph without any need to be considered as a formal taxon. Within the Droseraceae family, AV with 'snap-traps' is sister to *Dionaea muscipula* and both are sister to the genus *Drosera* (Elansary et al., 2010; Cross, 2012; Fleischmann et al., 2018a, 2018b).

Recent molecular clock estimates date the split of the snap-trap *Dionaea* + *Aldrovanda* clade from *Drosera* at 53.4 million years ago and the split of *Dionaea* and *Aldrovanda* lineages at 48 million years ago (Fleischmann et al., 2018b). The fact that *Dionaea* and *Aldrovanda* are sister genera has high molecular and morphological support and is also corroborated by the fossil evidence that AV also occurred in North America (Alaska) during the Late Tertiary (see Fleischmann et al., 2018a). Generally, 19 fossil *Aldrovanda* species have been described from all continents but South America, but *A. vesiculosa* is the only extant species that has survived to recent times (Cross, 2012; Fleischmann et al., 2018a).

2.3. Flowering and reproduction

Similar to many aquatic plants relying mainly on vegetative propagation, AV displays reduced generative reproduction (Cross, 2012). Although the flowering of temperate AV populations at natural European sites or in outdoor cultures is generally accepted as rare and poorly successful, botanical and herbarium records indicate that AV can flower in virtually all of the European countries and regions where it occurs (from Italy to NW Russia near Lake Ladoga) and temperate populations in Japan or the USA (introduced here) as well as (sub)tropical ones in Africa and Australia also do so (Adamec and Tichý, 1997; Okada, 2008; Cross, 2012; Cross et al., 2015, 2016). On the basis of all these studies on various AV populations, it can be concluded that the flowering of temperate populations is initiated by optimum ecological factors such as a longer period (3–4 weeks) of high water temperature (ca. > 26 °C), high irradiance, adequately high CO_2 concentration and prey availability. As an example, the flowering of AV plants originating from the Hungarian population at an artificial site in S Bohemia, Czech Republic, was preceded by five days of high surface water temperature at a maximum of 28 to 33 °C (Cross et al., 2016). Despite this, AV flowering is considered very variable between seasons and populations and seed set even much more variable as the percentage of fertile capsules may be zero (cf. Adamec and Tichý, 1997; Adamec, 1999c; Cross et al., 2016).

A solitary flower bud is initiated at the shoot apex under water (Okada, 2008; Cross, 2012). During its development, its erect flower stalk is enlarged to 12–16 mm and reaches the water surface. It is developed on a modified leaf whorl devoid of traps and their petioles contain large air lacunae. The shoot in the point of the flower stalk is also partly bent downwards. Both adaptations help to stabilise the flower near the surface. Mature flowers emerge slightly above the surface only at anthesis when they open (Fig. 2). The open flowers are radially symmetrical, pentamerous, tetracyclic, bowl-shaped, and possess five green or reddish sepals and five petals 3–5 mm long (Okada, 2008; Cross, 2012). The petals are white in all temperate populations including the red Hungarian one, but are slightly rose in red Australian populations (Cross, 2012). The whole corolla may be 6–8 mm across in temperate plants, but up to 10–12 mm in African or Australian plants (Cross, 2012). The hermaphrodite flower has a subglobose, superior ovary which bears five whitish styles radially alternating to five slender stamens with yellow anthers (Fig. 2). The stigmas are covered with many distinct projections. It is important for the pollination that some flowers have straight styles making no contact with anthers, while in others, some styles are bent towards the anthers (Okada, 2008; Cross, 2012). The pollen to ovule ratio (28.5 ± 4.6) suggests a strictly autogamous or self-fertilising nature which was also confirmed experimentally (Okada, 2008) and in closed aquaria without any access for

Fig. 2. Detail of the open flower of AV (from Hungary) at an artificial site in a sand-pit at Suchdol nad Lužnicí, S Bohemia, Czech Republic; August 2014.

Fig. 3. Ripe fertile capsules of AV (from Hungary) from an artificial site in a sand-pit at Suchdol nad Lužnicí, S Bohemia, Czech Republic; August 2014.

insects (L. Adamec, unpubl. data). A fly as a flower visitor was described in Japan but its role for AV pollination is unknown (Okada, 2008).

According to the stage of floral development, two distinct types of flowers can be distinguished in AV: (facultatively) autogamous (open) and cleistogamous (closed; Adamec and Tichý, 1997; Adamec, 1999c; Okada, 2008; Cross, 2012; Cross et al., 2016). All available pieces of evidence confirm that seed set can only occur in opened flowers. It is hypothesised that AV pollen longevity is limited to only a few hours and that pollen is active for a few hours only after the flower has opened (A. Moscatelli and M. Beretta, unpubl. data). As confirmed repeatedly both at natural sites and in outdoor cultures in temperate AV populations, flowers open fully only for several hours (2.5–3 h), usually in the afternoon when water temperature is highest (Degreef, 1986; Adamec and Tichý, 1997; Okada, 2008; Cross, 2012; Cross et al., 2016). High afternoon water temperature (ca. > 27 °C) is considered the key ecological factor regulating flower opening and thus seed set. At an artificial AV site in Virginia, USA, the afternoon water surface temperature correlated with the number of open flowers (Cross et al., 2016). The anthers open just after the petals opened and each anther (per stamen) contains 12–22 pollen tetrads (Okada, 2008). The open flowers with their petals nearly floating on the surface are very susceptible to any mechanical disturbance (wind, water waving, rain, fish or bird movement) as they can very easily become submerged (Adamec, unpubl.) and, probably, their pollen grains are lost. Very warm and calm weather is thus a prerequisite for a successful AV pollination and seed set. On the other hand, under suboptimal (temperature) conditions, only cleistogamous flowers with abortive yellowish capsules are formed (Berta, 1961; Adamec and Tichý, 1997; Cross, 2012). Fruiting success is highly variable and depends on local conditions: from 0 to 80–90% (Adamec and Tichý, 1997; Adamec, 1999c; Okada, 2008; Cross, 2012; Cross et al., 2016). At three AV sites on two continents, ca. 81–94% of all initiated flowers resulted in infertile fruit and abortive flowers (Cross et al., 2016).

After pollination, the flower closes again and the flower stalk reflexes under water (Fig. 3). Development of ripe capsules takes place in 2–4 weeks (Cross, 2012). Unlike pale abortive capsules, ripe fertile ones are larger, 3–5 mm long and 3–4 mm wide, and dark green or green-red with thin membraneous walls (Fig. 3; Cross, 2012). The shape of capsules from different populations can be variable (Adamec, 1999c; Cross, 2012). The resistant fertile capsules with stalks are shifted to senescent shoot bases after several weeks. After separation from the plants, they

can float at the water surface for several weeks until the capsules rot and the seeds are released and sink down (Adamec, 1999c; Cross, 2012). The number of ripe, hard seeds per capsule is also highly variable both between sites (or cultures), populations or seasons and within populations (Adamec, 1999c; Cross, 2012; Cross et al., 2016). The number of ripe seeds per capsule ranges from 1 to 14 across continents or sites, but the mean or median is usually only 4–6 (Adamec and Tichý, 1997; Adamec, 1999c; Okada, 2008; Cross, 2012; Cross et al., 2016). Ripe ovoid and rigid black seeds are 1.2–1.6 mm long and their mean weight is 0.82 mg (Cross, 2012; Cross et al., 2016, 2018). Their exotesta contains complex honeycomb-like channels 10–20 μm wide covering palisade-like endotesta with small pores of 1–3 μm. These structures reduce the density of the seed and could assist in gas exchange (Cross et al., 2018). A small, capitate embryo sits below an operculum.

The germination rate of AV seeds can be extremely variable. Older fragmentary items of knowledge on AV's germination traits (see Cross, 2012 and the references therein) have recently been complemented by Cross et al. (2016, 2018). Generally, AV seeds exhibit a physiological dormancy. The optimal germination temperature for freshly collected seeds from SW Australia was 25 °C in light, while no seeds germinated in darkness at any temperature. Seeds also germinated best with ethylene present and after an 8-week cold stratification (at 5 °C) interrupted by an 8-week warm period at 25 °C. However, the temperature range for germination was very narrow. Gibberellic acid had no effect on germination. Ethylene is produced by microbes in the organic sediment on the bottom in response to wetting and drying events and AV seed germination probably happens in the spring or summer periods after the water temperature rises following winter stratification and irradiance increases (Cross et al., 2016, 2018). The ethylene stimulation of seed germination thus leads to significant population recruitment from the seed bank after the re-flooding of dried sediments. In a germinating seed, the root at its maximum length of 2–3 mm protrudes from the operculum but it stops growing afterwards (Cross, 2012). The cotyledons remain inside the seed and support the growth of the seedling.

Controversial views exist on the longevity of AV seeds and on the existence of a seed bank (Cross et al., 2016, 2018). As reported by the former authors, when fresh seeds in bags were buried in sediment 1 cm deep at a natural Australian site for 6 months, only 20% of the seeds survived and all seeds died after one year of burial. In a similar seed bag experiment on AV plants of Hungarian origin at a shallow artificial site in the Czech Republic, only 3–7% of seeds (exposed on the top soil or buried 1–2 cm into the sediment) were damaged after 11 months

(Adamec, 2018b). There was no marked difference between both variants. Moreover, the seeds were able to germinate at 20–21% rate after one year changing aquatic exposure in a refrigerator or outdoors. In line with this, population recruitment occurred after a break of several years when a lake dried out at a natural Hungarian site (L. Adamec, unpubl. data). This confirmed a functional seed bank surviving for several (ca. 10) years. Seeds stored in a dry state (15 °C and 15% relative humidity) had a partially reduced germination rate after one month, but completely failed after one year. Similarly, freezing fresh seeds to -18 °C for only 24 h reduced the germination rate by 10–45%, while freezing for three months completely blocked germination (Cross et al., 2016).

In total, 1–5 solitary flowers (flower buds) can be produced per plant (Adamec and Tichý, 1997; Cross, 2012; Cross et al., 2016). The rate of production of flower buds on one plant is similar to that of the branching rate: on average, 5.0–6.4 stem internodes separated two successive flowers in flowering Czech AV populations (L. Adamec, unpubl. for the data in Cross et al., 2016). Assuming a high apical shoot growth rate of 1–1.2 leaf whorls/day, a new flower bud is initiated every 4–6 days. Initiation and development of flower buds influence the growth of the shoots and branching frequency (Cross et al., 2016). The pooled data from six AV sites on three continents showed that the number of flowers per plant correlated negatively with shoot length (P = 0.022), but positively with number of mature whorls (P = 0.002). Moreover, the number of flowers per plant correlated negatively with the number of branches (P = 0.032) suggesting a strong competition between flowering and vegetative propagation. At European sites, the first signs of flowering of AV occur at the beginning of July, flowering peaks in mid-August, but the last flowers are initiated as late as at the second half of September (Degreef, 1986; Adamec and Tichý, 1997; Cross, 2012; Cross et al., 2016) when turion development begins. It is therefore common for ripe turions to contain a miniature abortive flower bud at their base. In conclusion, although all of world's AV populations can flower and also set fertile seeds, the ecological importance of seed-set presumably only consists of the formation of the seed bank and in the dispersal (zoochory) to new sites (Cross, 2012; Cross et al., 2016). During the growing season, the plants propagate only vegetatively (clonally) by branching (Adamec, 1999a).

2.4. Overwintering and turion biology

Turions are vegetative dormant winter buds formed by aquatic plants as a response to unfavourable ecological autumnal conditions. They protect fragile summer shoots from freezing and inclusion in ice (for review, see Adamec, 2018c). All known temperate populations of AV form turions as overwintering organs; turion formation also surprisingly occurs in some Australian (sub)tropical populations (Adamec, 1999a, 1999b, 2003, 2018a, 2018c; Cross, 2012, 2013). Turions of temperate AV populations are obtusely rhomboid and slightly tough organs formed by the strong condensation of modified leaves and very short internodes in shoot apices at the end of the growing season (Fig. 4; Adamec, 1999b, 2003; Cross, 2012). They are 5–8 mm long, 3–6 mm wide and dark green due to high chlorophyll content. Unlike the shoot apices of growing summer plants with long and conspicuous bristles, true dormant turions do not look 'hairy' (cf. Figs. 1 and 4). Turions of AV are formed in response to a marked and longer decrease in water temperature combined with reduced irradiance, while shortening of the day length itself is not important (Adamec, 1999b, 2018a). In Central Europe, turion formation in the field starts at the end of August to mid-September. As shown repeatedly in an outdoor collection, a northern European AV population (Lake Ladoga, NE Russia) starts forming turions ca. 3–4 weeks earlier than more southern European or Japanese populations (Adamec, 2018c). The onset of turion formation in various populations is thus genetically fixed.

At artificial Czech AV sites and also in the Třeboň outdoor culture, turions fully ripen in the latter part of October as low temperatures of ca. < 6 to 8 °C are essential for their physiological ripening (Adamec,
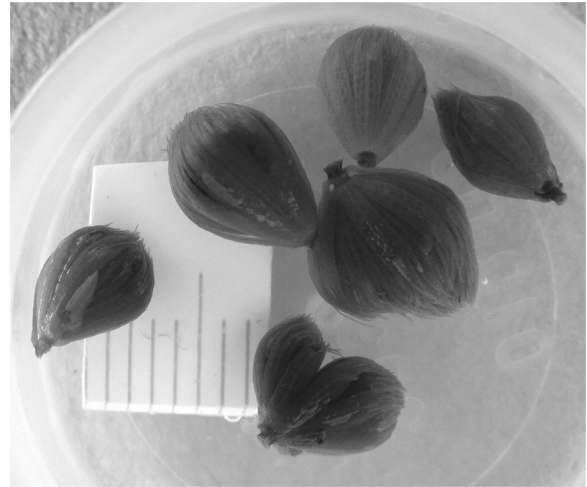

Fig. 4. Turions of AV (from E Poland) stored in a refrigerator over winter; February 2018. Note that turions are very condensed and their bristles are not reflexed. Ticks represent 1 mm.

1999a, 2018c). By the end of October, ripe turions either break off from mother shoots by an abscission layer and sink to the bottom to overwinter, or unseparated turions are dragged down by the decaying mother shoots (Adamec, 1999a, 1999b). All temperate *Aldrovanda* populations are able to form the abscission layer under suitably cold conditions (Adamec, 2018c). Most turions float to the surface and start sprouting from the end of April to early May (Adamec, 1999a, 1999b). The survival rate of overwintering turions at the Czech sites was 0–70% (mean ca. 25–40%; Adamec, 1999a) and ca. 19–100% in a similar study (Adamec and Lev, 1999). The losses were partly caused by grazing of the ripening floating turions by ducks or the emergent ripe turions were grazed by small rodents. However, no emergent turions died due to strong frosts (Adamec, 1999a, 1999b). In conclusion, turion overwintering at stable natural AV sites results in considerable population losses which must be compensated for by rapid seasonal vegetative propagation. Moreover, these turion costs are also offset by the phenological benefit as sprouting turions can grow rapidly at the surface in the warmest water and at maximal irradiance in combination with their storage function, thus somewhat extending the AV's short growing season. At very shallow AV sites, a variable fraction of turions can overwinter freely exposed directly on wet substrate where they face drought and frost conditions (Adamec, 1999a, 1999b). AV turions are extremely sensitive to drought and cannot survive even a short drought period (Adamec, 2008c). Non-hardened AV turions stored in a refrigerator were very sensitive to frosts of ca. −8 °C but their frost resistance was markedly increased after their outdoor winter hardening by natural weak frosts (Adamec and Kučerová, 2013a). Generally, the frost avoidance in non-hardened turions was changed to frost tolerance.

Controversial data exists on turion formation in various AV populations from the southern hemisphere (cf. Adamec, 1999b, 2018c; Cross, 2013). Adamec (2005b) published wrong preliminary data on turion formation in African plants from Okavango Delta, Botswana, which has been accepted and cited by Cross (2013). Out of six extant and cultivated Australian AV populations, only plants from a subtropical montane site near Armidale in N.S.W. and tropical ones from Katherine in N.T. clearly formed true dormant turions in the collection in the Institute of Botany at Třeboň, Czech Republic, while the remaining four populations formed only non-dormant or weakly dormant, quiescent winter apices with long bristles, both indoors and outdoors (Adamec, 2008b, 2018c). However, Cross (2013) states that turion formation occurs in all six Australian populations. In some populations, it may be difficult to distinguish between true dormant turions and non-dormant winter apices unless a measurement of dark respiration rate as a crucial criterion for dormancy is conducted (Adamec, 2008b).

Regardless of the dormancy *sensu stricto* or non-dormancy, tropical AV plants from Katherine and Darwin, N.T., successfully overwintered experimentally under ice at two Czech artificial AV sites (L. Adamec, unpubl.). Moreover, dormant turions are not formed in all nearly monoclonal plants within the Armidale and Katherine populations every season, suggesting that epigenetic modifications take place (L. Adamec, unpubl.).

Analogous with turions of other aquatic plants, two dormancy stages – innate and imposed dormancy – were recognised in temperate AV turions (Adamec, 1999b, 2003, 2018c). The innate dormancy starts with turion ripening and is broken around mid-January, being replaced by an imposed dormancy until the turions germinate and sprout in warmer water at > 12 °C. Breaking the imposed dormancy due to this temperature rise is associated with the spring floating (buoyancy) of the turions to the surface. Turion floating is caused by an increasing proportion of gas volume in leaf lacunae in the inner turion leaves from ca. 10–20% to nearly 100%, probably as a result of increased aerobic respiration or anaerobic fermentation rates (Adamec, 2003, 2018a, 2018c). Turions of AV also function as storage organs: in autumn, they accumulate starch, free sugars, lipids and reserve proteins and their total content of carbohydrates becomes markedly reduced over winter (Adamec, 2000, 2003, 2018c; Płachno et al., 2014). They also store mineral nutrients (N, P, Mg, S), although this is apparently less important (Adamec, 2010, 2011b). The turions exhibit low metabolism intensities and their dark respiration rate at 20 °C per unit fresh weight (FW) is about 2–3 times lower (2.7-4.5 mmol $kg^{-1} h^{-1}$) than that for summer shoots (Adamec, 2003, 2008b, 2011b). The temperature quotient Q_{10} for dark respiration (between 4 and 20 °C) was 1.75 in innately dormant turions and this significantly increased to 2.94 in imposed dormant ones (Adamec, 2008b). In line with this, the anaerobic fermentation rate of AV turions was only 1.5–7% of that of aerobic respiration (Adamec, 2003). Moreover, even under optimal conditions, turions exhibited a negative photosynthetic rate, while that of sprouting turions was very high and comparable with that of summer shoots (Adamec, 2011b). In conclusion, in spite of the very low rate of respiration/fermentation, the natural survival of AV turions is confined only to the next season (Adamec, 1999a, 2003, 2018c). Turions of AV thus do not form a type of 'seed bank'.

3. Distribution and habitat requirements

3.1. Geographical distribution: natural distribution

The sparse natural historical distribution of AV on four continents included only 379 sites (Cross, 2012) but spanned almost 100° of latitude from NW Russia to SW Australia (Berta, 1961; Kamiński, 1987a; Adamec, 1995; Weber, 1995; Cross, 2012; Fleischmann et al., 2018a). In summary, Cross (2012) reports only 50 extant, verified natural and 145 unverified (last record > 10 years ago) AV sites in four continents of the Old World; 184 historical sites are now extinct. He also reports 28 extant artificial sites in Europe and the USA which have arisen by the introduction of plants to new sites. Generally, AV always exhibited an extreme disjunction of its distribution and many populations were isolated from each other by hundreds or even thousands of km. All authors dealing with the world distribution of AV suggest that the distribution is caused by water birds as vectors covering these long distances. In Australia, six AV sites (or small groups of sites) have been described since 1995 and plants from these six sites are kept in the collection of the Institute of Botany CAS at Třeboň, Czech Republic (Elansary et al., 2010; Cross, 2012). It is probable, however, that some small sites are now (in 2018) extinct but some others could have arisen close to the older ones (Cross, 2012). As almost all Australian AV populations are generally much smaller in population size than temperate European ones, it renders them much more vulnerable. AV has been reported as extinct in East Timor (Cross, 2012).

The AV distribution in Africa has been very poorly surveyed.

Although AV was historically found in at least 14 countries (Botswana, Burundi, Cameroon, Chad, Ghana, Malawi, Mozambique, Rwanda, South Africa, South Sudan, Tanzania, Togo, Uganda, Zambia) and at 32 sites therein, the recent occurrence (after ca. 2005–2010) has been confirmed only in two countries: Botswana (several large sites in the Okavango Delta) and three sites in Uganda (Kamiński, 2006; Cross, 2012; Kalema et al., 2016).

According to Cross (2012), AV was historically known at 27 sites in nine Asian countries (Bangladesh, China, India, Japan, Kazakhstan, North Korea, South Korea, Russia - Asian part and Uzbekistan). However, AV has been reported extinct in Bangladesh, India and Uzbekistan and has not been confirmed in NE China (Manchuria near the Ussuri river), Kazakhstan, North Korea and South Korea in the last 10–15 years (Cross, 2012). However, a new AV site with red plants was discovered in a shallow lake dominated by *Phragmites australis* in the Qixinghe National Nature Reserve in NE China in 2017 (Y. Yunlong, pers. observ.). In 2018, ca. 4000 plants was estimated at the site. In the Russian Far East close to the Chinese and North Korean border, near the town of Khasan in the Primorsk county, an abundant AV site in nymphaeid-dominated Lake Lotos was discovered in 2016 (www.plantarium.ru/page/image/id/542035.html) and verified in August 2018 (P. Volkova, pers. observ.). The plants are also red. In Japan, all 12 historical sites have been destroyed (Cross, 2012) but two populations (near Hanyu and near Tatebayashi) have been restored as a result of conservation and management using the original cultivated populations (Y. Katagiri, pers. comm.).

In spite of the extensive habitat degradation and the loss of shallow wetlands in Europe during the last two centuries (water eutrophication, drainage, infilling of water bodies, afforestation and intensive agriculture; Walters, 1979; Adamec, 1995; Kamiński, 1987a, 2006; Cross, 2012; Fleischmann et al., 2018a), Europe – mainly Central and Eastern – still represents the centre of the world's extant AV distribution. On the basis of the most recent data, Cross (2012) states that 38 extant natural AV sites in Europe survived, but 90 are unverified (for > 10 years, probably extinct) and up to 164 are extinct. Overall, AV has vanished from at least six countries (Austria, Czech Republic, Slovakia, Germany, France and Italy) during the last century and its extant occurrence in Croatia, Montenegro, Latvia, Estonia, Serbia and the European part of Turkey is unverified and improbable. Extant natural populations thus only occur in Belarus, Bulgaria, Greece, Hungary, Lithuania, Macedonia, Poland, Romania, Russia and Ukraine (Fig. 5; Cross, 2012).

AV probably vanished from the last Austrian site at the beginning of the 20th century (Cross, 2012). In the Czech Republic, the only site near the Polish border close to former sites in Polish Silesia was verified in 1952 and extinct in the later 1950s (Adamec, 1995). AV from the only site in SE Slovakia vanished in 1984 (Adamec, 1995). In Germany, the last site in Lake Heege near Sperenberg south of Berlin vanished by the end of the 1980s (Adamec, 1995; Weber, 1995). The last French sites near Lacanau in SW France were extinct in 1969 or by the 1970s (Adamec, 1995; Cross, 2012). At the last Italian site, Lake Sibolla near Lucca, NE of Pisa, AV persisted up to 1997 when the habitat suffered from eutrophication and low water level (Adamec, 1995; Cross, 2012).

In Poland, at least 79 historical sites of AV occurred during the last two centuries and have been recorded in the literature (Kamiński, 1987a, 2006, 2014, unpubl. data of 2006–2016; Adamec, 1995; Cross, 2012). Therefore, Poland may be regarded as the country both with the greatest number of historical sites and the greatest density of them in the world. Such a prolific historical spread of AV in Poland was possible thanks to thousands of smaller shallow lowland lakes of a dystrophic or peaty character as potential sites (Kamiński, 2006, 2014). From the historical spread, AV occurred mainly in four metapopulations in lake districts comprising 8–44 sites each and the distance between the neighbouring sites was usually only a few km (Kamiński, 2006, 2014; Cross, 2012). They were: the Łęczna-Włodawa Lake district in E Poland (which neighbours a group of Ukrainian sites near Shatsk), the Augustów-Suwalki Lake District in NE Poland, the upper Odra river basin
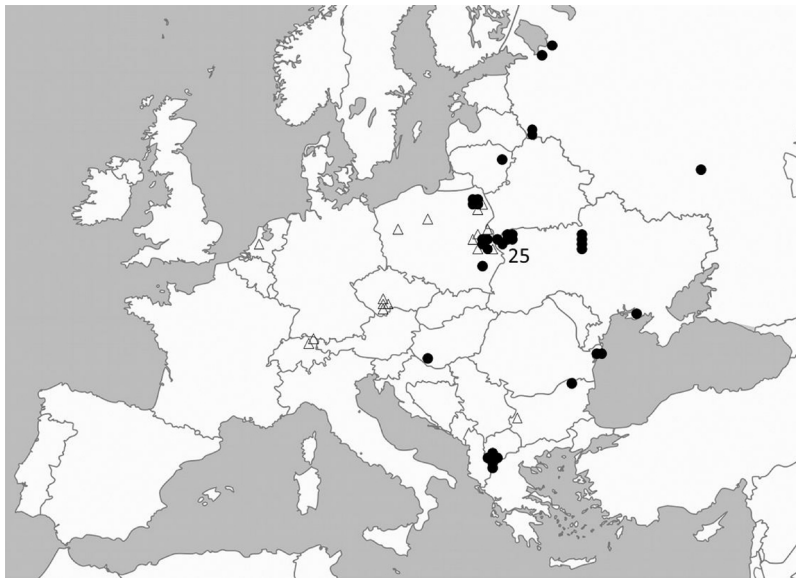
Fig. 5. European map of recent distribution of AV. Circles, natural sites confirmed recently after 2005-2010. The group of natural sites in NW Ukraine includes 25 sites. Triangles, artificial sites originated as a result of introduction and confirmed as extant after 2010-2015.

in Silesia in S Poland and the Gniezno Lake District in central Poland. The other sites were scattered in small groups containing at most three water bodies. However, in 1983, only 14 verified sites existed in Poland (Adamec, 1995).

Recently, only nine natural sites with a continual AV occurrence, which were verified between 2006–2013, were reported from Poland (Fig. 5; Kamiński, 2006, 2014, unpubl. data of 2006–2016). Within the Łęczna-Włodawa Lake District in E Poland, AV grows naturally at four sites in Lakes Długie, Moszne, Sumin and Mytycze (or Motycze). Additionally, as a result of the successful reintroduction of plants from local populations in this area conducted after 1998, AV occurs in another five former historical sites in Lakes Płotycze, Łukie, Perespa (Pereszpa), Koseniec and Uściwierz. Within the Augustów-Suwalki Lake District in NE Poland, AV grows naturally at four sites in Lakes Mikaszówek (named also Miklaszówek or Miklaszewo) and Kruglak, in a widened part of the Augustowski Canal near Lake Mikaszówek and in the Augustowski Canal in front of its inlet to Lake Krzywe. In 2012, a new abundant AV site was discovered in a small lake in Średnie Duże village near Zamość in Lubelska Highlands in SE Poland (Kamiński, 2014). Out of these 14 natural AV sites (both with continuous and discontinuous occurrence), six sites recently hosted > 50,000 plants and three even > 100,000 plants (R. Kamiński, unpubl. data of 2006–2016). Many attempts to introduce AV to potentially suitable sites in areas (lake districts) of its former historical range in Poland within 1992–1998 were undertaken (Kamiński, 2006, 2014, unpubl. data of 2006–2016). Out of a total of eight reported introductions, only four of them may be recently considered successful (Fig. 5): Lakes Chylinki (Chylniki) and Krejwielanek (Krzywulek) in the Augustów-Suwalki Lake District in NE Poland, Lake Święte near Miały in W Poland and Bagno Mostki peat bog pools near Bachotka in the Brodnickie Lake District in central Poland. At these artificial sites, a population size > 1000 plants was reported from 2004 to 2013 (R. Kamiński, unpubl. data of 2006–2016).

In conclusion, 14 natural and four artificial extant AV sites have been reported on the basis of recently-available data in Poland. However, nine of these 18 sites have been created either by a re-introduction to historical sites or an introduction to other sites in areas covering the historical range of AV. The comparison of AV distribution or population sizes at single sites in Poland over a longer time of one-two decades clearly shows that AV is a 'jumping species' with highly fluctuating population size, which can lead easily to its extinction and

distribution changes.

In Lithuania, three historical AV sites are extinct but a new very abundant site was discovered in Lake Ruzhas near Utena in E Lithuania in 2001 (Vilkonis, 2003). In Belarus, in total 22 historical AV sites in three regions are reported but they all are presently unverified (Kamiński, 2006; Shiyan and Andrienko, 2011; Cross, 2012). Shiyan and Andrienko (2011) evaluated herbarium data on the historical records of AV from the Ukrainian-Belarusan border regions of the Pripyat (Pripet) river basin and documented 13 historical sites on the Belarusan side. They have confirmed recent extinction at nine sites, while the others were not verified. The highest concentration of historical sites in this area of Pinsk (or Pripyat) Marshes was in the Pripyat river basin near Turan and Pinsk and also in the SW tip of Belarus near Shatsk. Nevertheless, in spite of no recent verification of an extant AV site in Belarus, given the many historical records, the presence of thousands of potential AV sites in dystrophic lakes in Belarus and also the occurrence of many extant sites (especially in NW Ukraine; Fig. 5), AV occurrence is highly probable in S, NW and N Belarus.

Ukraine has (together with Poland and Belarus) the highest number of historical AV sites in Europe approaching ca. 45–50 (cf. Kamiński, 2006; Shiyan and Andrienko, 2011; Cross, 2012). Shiyan and Andrienko (2011) searched for historical AV sites in NW Ukraine (Volynska and Rivnenska counties) in the Pripyat river basin near the border with Belarus in 2001–2011. Out of the total 33 historical sites in this area, AV was confirmed at 25 sites on an area of around 3000 km^2 (Fig. 5). The extant sites were mostly concentrated in the basin of the Stokhid river near Loknytsya, in the Vyzhivka river basin near Stara Vyzhivka and in the basin of the upper reaches of the Pripyat river near Shatsk. However, only two sites in other Ukrainian regions were confirmed after 2001. An extremely abundant AV site counting millions of plants and extending over a few km was reported on the western bank of the Kiev reservoir (on the Dnieper river) at the estuary of the Teterev river near Stracholes'e village south of Chernobyl in N Ukraine in 1997 (Elansary et al., 2010). This site and three other abundant ones were also confirmed in extensive bays or shallow wetlands on the western bank of the Kiev reservoir near the villages Sukholuch'e and Tolokun' about 10–16 km south of Stracholes'e, during 1998–2000 (V. Rakov, pers. comm.). In S Ukraine, AV was discovered in the extensive Dnieper delta in Kardashynskyi Lyman Lake near Kherson in 2001 (Kamiński, 2006). Similar to Belarus, AV in Ukraine might still be more abundant due to thousands of backwaters in the basins of large rivers (Dnieper,

Dniester, Desna, Juzhnyi Bug).

In spite of the vast territory of the European part of Russia and around 20 historical AV sites in this area, only five of them have been reliably verified in three different regions since 2003 (Fig. 5). Afanas'ev (1953) discovered an extremely abundant site in a shallow, *Phragmites australis*-dominated lake near the estuary of the Sviri river on the southeastern bank of Lake Ladoga in NW Russia. It represents the northernmost world site (at 60.5 °N!) but simultaneously also one of the most abundant world populations of this species as its dense stands occurred along a 2 km long bank of the lake in the 1990s (Adamec, 1995). The abundant site was confirmed also in 2000–2005 (L.E. Muravnik, pers. comm.). Recently, a new abundant AV site has been found near the southern bank of Lake Ladoga, about 108 km southwest from the first one, in a shallow lake 3 km south of Kukkorevo village (Doronina, 2014). Since 2005, two close-by sites have been found in the tip, very close to the Latvian and Belarusan border in the Pskov county in W Russia. In 2005, AV was found in the dystrophic Lake Berezvica (A. Ivanova, pers. comm.), while another site in Lake Dolgo'e (ca. 20 km from the latter) in the Sebezh National Park was found in 2016 (A.J.Doronina, pers. comm.) and the plants are grown in the Institute of Botany at Třeboň. In 2003, a new abundant AV site was discovered in an extracted peatbog in Lake Mokhovo'e in the Galich'a Gora Nature Reserve near Soshki village in the Voronezh river basin about 25 km south of Lipetsk in the Lipetsk county in SW Russia (A. Slavgorodskii, pers. comm.) and the plants are also grown in the Institute of Botany at Třeboň. Around 2013, another AV site (Chistyi prud) was discovered in this area, but the site was dry and extinct in 2017 (A. Grigorov, pers. comm.). Based on historical sites (Berta, 1961), AV could still occur in the extensive Caucasus region, in a Don river basin in the Rostov county and in the Kuban' river basin in the Krasnodar county; but recent verifications are missing (Kamiński, 2006; Cross, 2012).

In Romania, 17 historical AV sites in five regions were described, but the only extant sites are in the Danube delta (Kamiński, 2006; Cross, 2012). In 1998 and 2007, abundant populations were verified at two neighbouring sites near the middle Sulina branch in the delta (Elansary et al., 2010; Cross, 2012). Due to hundreds of potential sites and low accessibility within the delta, it is possible to expect that the true number of extant sites may be much greater there. In Bulgaria, only two historical AV sites are reported from different regions (Kamiński, 2006; Cross, 2012). In 1988, AV was found in Lake Srebarna in NE Bulgaria and the population existed in 2011 (V. Ralev, pers. comm.). The large shallow lake situated about 2 km south of the Danube river and ca. 18 km west of Silistra is the core of the Srebarna Nature Reserve. AV historically occurred in the Dragoman Marsh, an alkaline fen complex in a karst area near Dragoman 700 m a.s.l., ca. 40 km northwest of Sofia in W Bulgaria, where the species was extinct after drainage in the 1960s (Berta, 1961; Kamiński, 2006; Cross, 2012). In 2007, AV plants from the Danube delta were reintroduced to some fen pools in the complex and the population size increased to around 6000 plants in 2011 (I. Hristov and V. Ralev, pers. comm.). Cross (2012) reports one extant and eight extinct historical sites of AV in Serbia. The only extant Serbian site could be Zasavica Nature Reserve, a complex of backwater oxbows and canals in the Sava river floodplain about 5 km west of Sremska Mitrovica and ca. 70 km NW of Belgrad in NW Serbia. AV was found here first in 2005 and in 2006, was confirmed in a 7 km long reach of the oxbow. Although it was also confirmed from 2008 to 2011 (Cross, 2012), P. Denčić (pers. comm., 2017) recently visited the site and reported the extinction. It is likely that AV was extinct as early as in 2009 or 2010 but its occurrence was mistakenly reported later on. Obviously, a thorough recent verification is needed here. AV has recently been rediscovered in Lake Prespa (853 m a.s.l.) in S Macedonia (Jovanovska et al., 2017). Four sites occur in the northern part of the lake between Sit Han and Asamati villages ca. 10 km along the shore and one site near Nakolec village on the eastern shore. All sites are situated in the shallow *Phragmites australis*-dominated littoral belt but the AV population is threatened by marked water level fluctuations and low water

levels. The total reed bed area along the Macedonian shore, which represents a potential habitat for AV, is estimated to be ca. 3.2 km². Five sites in Lake Prespa were verified in July 2018 (D. Jovanovska and L. Adamec, pers. observ.). In 2011, AV was also rediscovered in the Greek area of Lake Prespa (Cross, 2012; Jovanovska et al., 2017).

In Hungary, six historical sites were reported from different parts of the country but only one is extant: the peaty Lake Baláta-tó in Somogy county in SW Hungary (Berta, 1961; Cross, 2012). Before 2003, a very abundant population boasting probably over one million reddish plants (var. *rubescens*) was growing there in *Phragmites-, Typha-* or *Carex-*dominated stands on an area of several hectares (Adamec, 2005b; F. Kasza, pers. observ.). However, in the summer 2003, due to extreme drought, only a few dozen of the last plants were found in the lake (L. Adamec, pers. observ.) and the population died afterwards (M. Toldi and F. Kasza, unpubl. data). The lake was more or less dry between 2003-2009. From 2010, the water level in the lake partly recovered and the reintroduction of plants from the original population occurred in 2011 and 2012 but these failed due to low water level (M. Toldi and F. Kasza, unpubl. data). The population recovered in 2013–2014, likely from a native seed bank (Cross et al., 2016) and in 2017, a smaller population was confirmed in the lake (M. Toldi, pers. comm.). Another small AV site was found in an oxbow near Pocsaj on the Romanian border in E Hungary in 2002. However, as the region is threatened by eutrophication and the site has not been verified since, it is now likely extinct (Cross, 2012).

3.2. Artificial European and American sites

Alongside the extant natural distribution in 10 European countries, AV has been successfully introduced into several shallow dystrophic wetlands in four countries (Switzerland, Germany, Czech Republic and The Netherlands) and new artificial sites have arisen in regions where AV has never grown (Adamec, 1995, 2005a; Cross, 2012). In 1908, AV plants from Lake Constance (Bodensee) in Germany were successfully introduced in an extracted peat bog in the small Lake Mettmenhaslisee near Niederhasli, north of Zurich in NE Switzerland (Koch, 1950; Adamec, 1995, 2005a; Cross, 2012). Akeret (1993) described an AV population in Mägestlenweiher pool in an extracted peat bog situated near Hettlingen ca. 20 km southwest from Mettmenhaslisee. The AV was probably transferred here spontaneously by water birds. Each site contained thousands of plants and were last verified in 2012 (A. Nijman, pers. comm.). In around 2010, an artificial site of AV arose in an abandoned concrete pond in a former military area in Wahner Heide near the Bonn-Cologne airport in W Germany as a result of an introduction of AV plants probably by an amateur enthusiast (Gorissen, 2015). The shallow concrete pond of ca. only 200 m² is inhabited by aquatic and wetland vegetation (*Typha* sp., *Carex* spp.) and mimics a natural site with a few hundred AV plants (M. Horstmann et al., unpubl., 2017). However, only one plant was found in June and August 2018 (S. Poppinga and A. Fleischmann, pers. observ.). This site is likely to become extinct.

AV was first introduced to potential dystrophic sites in the Czech Republic in 1995 and in 2017, four extant sites of different size and nature occurred in the Třeboň basin in S Bohemia in the SW part of the country (Adamec and Lev, 1999; Adamec, 2005a; Adamec and Kučerová, 2013b; Cross et al., 2016; L. Adamec, unpubl. data of 2017). The largest and most abundant site is Karštejn, near Veselí nad Lužnicí representing a ca. 4 ha large complex of extracted fen lakes dominated by *Phragmites australis* and *Carex rostrata*. The total AV population size at the site fluctuates between ca. 50,000 and 200,000 plants and depends markedly on the water level. Various microsites are subject to overgrowing by a dense *Phragmites australis* stand. Another artificial site occurs in two nearby fen pools adjacent to the hypertrophic Ptačí blato fishpond, west of Lomnice nad Lužnicí. Both microsites have been subject to eutrophication and the gradual overgrowing by dense helophyte stands in combination with very low water levels (see Fig. 6). The

Fig. 6. Shoots of AV (from E Poland) growing on wet substrate due to low water level at an artificial site in a fen pool at Ptačí blato fishpond in S Bohemia, Czech Republic; June 2017.

Fig. 7. An artificial site of AV (from Hungary) in a deeper sand-pit pool at Suchdol nad Lužnicí, S Bohemia, Czech Republic; August 2017. All photos by L. Adamec.

population numbered ca. 10,000–20,000 plants during 1997–2014, but in 2017 and 2018, only 1000–2000 plants occurred. Branná sand-pit is a small, meso-eutrophic, dystrophic sand-pit pool about 60 m^2 large surrounded by forest near Třeboň. An AV population not extending ca. 100 plants faces strong competition with invasive aquatic vegetation (*Potamogeton natans*, *Hydrocharis morsus-ranae*, *Chara* spp.) and filamentous algae and is fully dependent on regular management. AV from Lake Długie from E Poland was introduced to these three Czech sites. In 2018, a very small, but formerly relatively abundant eutrophic site in a peaty pool on the margin of Výtopa fishpond near Lutová village (Adamec, 2005a; Cross, 2012; Adamec and Kučerová, 2013b) was extinct due to rapid infilling by reeds (L. Adamec, pers. observ.). In 2009, red Hungarian AV plants were introduced to two small (ca. 120 and 80 m^2), shallow, slightly dystrophic pools in a large complex of extracted sand-pit Cep I near Suchdol nad Lužnicí (Adamec and Kučerová, 2013b; Cross et al., 2016). These mesotrophic pools are inhabited by dense aquatic vegetation typical of soft waters (Fig. 7). AV populations in both pools peaked in 2014 or 2015 when ca. 5000–6000 plants occurred in each of them and the plants abundantly flowered and set seed (Cross et al., 2016). However, due to very dry 2015 and 2016 summer seasons, the water discharge through both pools markedly reduced or fell to zero and an unknown '*Aldrovanda* disease', probably of fungal origin, (Adamec, 2005b; Cross, 2012) has decimated the population since. In 2017, about 100 plants occurred in each pool (L. Adamec, unpubl. data). In 2006 and 2007, AV plants originating from E Poland

and Hungary were introduced to some shallow, *Phragmites australis*-dominated lakes in the Nature Reserve De Haak near Nieuwkoop ca. 20 km south of Amsterdam in The Netherlands (A. Nijman, pers. comm., Cross, 2012). In 2012, abundant populations numbering tens of thousands of plants overall were confirmed in some of the lakes (A. Nijman, pers. comm.; see also Creuwels and Pieterse, 2018).

Although AV has no natural distribution in North America, plants of two Japanese populations (from Hanyu and Kyoto) were introduced by carnivorous plant growers to several dystrophic lakes in Virginia, New Jersey and New York states in the USA in the 1980s and 1990s (Cross, 2012; Lamont et al., 2013). Since the AV populations were introduced they have experienced marked fluctuations in population counts and areas but the largest extant (2013) populations contain millions of plants. In Virginia, seven extant sites are in Caroline, Hanover, Louisa, Orange and Spot-sylvania counties. AV was introduced to 12 potential sites in New Jersey in 1999 but only one site (Lake Owassa, Sussex county) has survived for various reasons (mainly low water level, competition by aquatic plants, muddy water) after 2009 (Lamont et al., 2013). In New York, only one extensive site (Big Pond) existed recently. In 2012, its population size was ca. 25–30 million plants. Thus, abundant artificial sites have arisen in temperate regions on a continent where AV has never naturally occurred. However, there is no tangible evidence to date that even a dense AV population exhibits any negative impact on these habitats, such as by outcompeting any of the native aquatic species (Lamont et al., 2013). Long-term experience from the Czech Republic also shows that abundant artificial AV sites can occur in intensively agricultural landscapes (Adamec, 2005a).

3.3. Habitats and plant communities

AV can grow in a variety of habitats in shallow, standing dystrophic fresh waters, but also in very slowly-flowing waters. At natural sites (both extant and extinct) in Europe, it usually occurs in shallow dystrophic or peaty lakes, reed- or cyperoid-dominated shallow margins (littorals) of deep lakes or dam reservoirs, in dystrophic fishponds, natural pools in peat bogs and fens and also in areas formed after peat extraction. The plants can also grow in slowly-flowing canals connecting lakes or fishponds, and commonly also in floodplains and in the basins of large rivers in backwater pools and oxbows beyond the reach of the commonly-occurring floods (Berta, 1961; Kamiński, 1987a, 2006; Cross, 2012). Similar habitats also occur also in other continents with natural or artificial AV occurrence (Cross, 2012). Moreover, AV can also grow at artificial sites in shallow pools in extracted sand-pits (Fig. 7).

Unlike the generally-accepted stenotopic character of AV and the variety of its ecological requirements for successful population growth and survival (e.g., Kamiński, 1987a; Adamec, 1995; Cross, 2012), AV can grow in stable stands representing its ecological optimum in association with a surprising number of aquatic plant species of different ecological forms as (co)dominants; this applies both for natural and artificial sites (cf. Berta, 1961; Kamiński, 1987a, 2014; Adamec, 1999a; Cross, 2012; Cross et al., 2015, 2016). At ten natural Polish sites, AV commonly occurred in plant associations belonging to the phytosociological orders Phragmition, Magnocaricion and Nymphaeion (Kamiński, 1987a). For all European sites, AV stands can generally be very stable if they occur within or near to loose stands of dominant tall emergent vegetation, mainly of the species *Phragmites australis, Typha angustifolia, Carex rostrata, C. acuta, C. elata* or *C. acutiformis*. These loose dominants are able to form large-scale, extensive, uniform optimal niches (microhabitats, shelters) for AV extending over hectares, or small bays or depressions in lake littorals of areas of only ca. 0.1 m^2 among dense emergent vegetation (Kamiński, 1987a; Adamec, 1997b, 1999a; Cross, 2012; Cross et al., 2015). It is important that the loose dominants protect AV stands from the unfavourable effects of winds and water waves, which could cause drift of the AV plants to unsuitable, deep sites of open water; they do not excessively shade AV stands, either (Adamec, 1997b; Adamec and Lev, 1999; Cross, 2012). Moreover, the

dominants regularly produce slowly decomposable plant litter which gradually forms sediments at the bottom. This litter is the principal factor for maintaining optimum water chemistry for AV: it continuously releases CO_2, tannins, humic acids and mineral nutrients (Adamec, 1995, 1997b, 1999, 2011a, 2018a; Cross, 2012).

European AV stands – especially at slightly eutrophic, lowland sites – are commonly accompanied by many (co)dominant free-floating, rootless submerged and rooted floating-leaved aquatic plant species, which may share the same or a similar ecological optimum as AV but their ecological amplitudes are much wider. It follows from the numerous literature (e.g., Berta, 1961; Kamiński, 1987a; Akeret, 1993; Cross, 2012; Cross et al., 2016) that the most common species are: *Hydrocharis morsus-ranae, Lemna minor, L. trisulca, Spirodela polyrhiza, Riccia fluitans, Salvinia natans* (the last five species are typical of the association *Spirodelo-Aldrovandetum*; see Berta, 1961), *Utricularia vulgaris, U. australis, Stratiotes aloides, Potamogeton natans, Nuphar lutea* and *Nymphaea* spp.

In conclusion, typical European AV habitats in shallow wetlands are usually very large (dozens or hundreds of hectares) and apparently uniform, but the AV stands alone are often very limited in area to only hundreds or thousands of m^2 in small, narrow bays or depressions or along a very short part of the shoreline. This underlines the stenotopic character of AV with its very strict ecological requirements and confirms the high vulnerability of AV at these sites (Adamec, 1995, 2005a; Cross, 2012). Other typical features of many AV habitats are the relatively rapid and unidirectional ecological changes due to habitat succession, which is commonly accelerated by low water levels and an increasing trophic level (Kamiński, 1987a; Adamec, 1995, 2005a; Cross, 2012). These dynamic changes of AV microhabitats can lead to extinction of local AV stands, but as a result of these habitat changes, new suitable microhabitats can continuously arise in these large habitats and AV stands can move dynamically with time. This habitat development can be best documented in the E Polish AV sites: plants were extinct in Lakes Płotycze and Łukie in the 1960s but after a re-introduction in the 1990s, very abundant populations have arisen (Kamiński, 2006, 2014; Cross, 2012).

3.4. Ecological requirements

Adamec (1995) summarised and listed 14 ecological requirements for European AV populations. These were based on the limited literature data on AV ecology and from his own extensive studies of the ecological factors at many natural Polish sites together with his experience of growing AV in artificial outdoor cultures. Recently, the original list has been partly modified by including data from many newer ecological studies (e.g., Adamec, 1997b, 1999a, 2005a; Adamec and Lev, 1999; Adamec and Kovářová, 2006; Adamec et al., 2010; Cross, 2012; Cross et al., 2015, 2016). Here, the ecological requirements are ordered according to their apparent importance: some are direct and principal but they are often interrelated.

1) As AV is clearly a photosynthetic strict CO_2 user (Adamec, 1997b, 2008a, 2011a, 2018a; Adamec and Kovářová, 2006), a relatively high CO_2 concentration in the water (minimum ca. 0.1 mM, optimum ca. 0.3–1 mM) should occur permanently in AV stands to ensure rapid plant growth and vegetative propagation; it should never decrease below ca. 0.04 mM (cf. Adamec and Kovářová, 2006; Cross et al., 2016).

2) The optimal water level at AV microsites is 0.2-0.5 m but the plants can also grow well in much deeper water. Deeper microsites (0.5–1 m) can be cooler but better resist eutrophication and overgrowing by dense reed or sedge stands; they also possess a greater reserve against excessive water level decline within the common yearly fluctuations. Very shallow water (5–10 cm) may support faster plant growth, but this also contributes to faster overgrowing by dense helophyte stands or filamentous algae and faster

eutrophication of the habitat, together with the increased risk of the habitat drying out. AV can survive well on wet substrate for weeks or even months but its growth stops (Fig. 6). Over winter, greater losses can be caused by rodents grazing the turions (Adamec, 1999a, 1999b). Generally, with yearly water level fluctuations, the water level should not decline < 10 cm. However, AV sites must not be flooded by a river as the majority of adult plants or turions could be washed away from the stand.

3) At the water surface, AV stands must be free of a dense biomass of submerged, free-floating and rooted floating-leaved macrophytes, filamentous algae and also of dead plant matter. As AV possesses a very low competitive ability it cannot produce a greater biomass under these conditions (Cross et al., 2015, 2016). However, loose stands of *Phragmites, Typha* or *Carex* spp. stabilise AV stands and represent the ecological optimum.

4) A thick layer (> 5 cm) of dead, nutrient-poor and slowly decomposing plant litter (of *Phragmites* or *Carex* or *Sphagnum* peat) should lie on the bottom of AV stands to optimise water chemistry (see above). This requirement can be limiting in shallow sand-pit pools. However, rapidly decomposable litter (e.g., of *Sparganium* spp., *Sagittaria, Glyceria maxima*) contributes to rapid eutrophication.

5) AV habitats should be rich in zooplankton or other potential prey as AV growth strongly depends on prey capture (Kamiński, 1987b; Adamec, 1999a, 2000, 2011a, 2018a). It is expected that enough prey can partly compensate for other unfavourable habitat factors (Adamec, 1999a).

6) AV stands should be sufficiently irradiated by the sun to ensure the high photosynthetic rate and the high water temperature required for its rapid growth. AV is relatively tolerant of shading and any irradiance within ca. 25–100% of that in the open is favourable, with an optimum of ca. 50% (Adamec, 1997b, 1999a).

7) Stand water should be relatively transparent, free of a large density of phytoplankton and fine suspended matter depositing on the plant's surface (Adamec, 1999a). Thus, the sediment should not contain suspendable fine fen particles but should be covered by plant litter.

8) A high temperature is necessary for rapid growth of AV and essential for flowering and fruiting (Adamec and Tichý, 1997; Cross et al., 2016). Over much of the growing season, water at the surface should be relatively warm, at least 16 °C on afternoons, with an optimum of ca. 26–30 °C. Nevertheless, if the historical spread of AV reached to the colder Atlantic region in NW Germany (Cross, 2012) and a new abundant site has recently arisen near the Atlantic coast in The Netherlands (Fig. 5), it is likely that maximum afternoon temperatures of only ca. 22–23 °C are quite sufficient for AV growth and propagation.

9) Low or medium concentrations of the nutrients (NH_4^+, NO_3^-, HPO_4^{2-}) typical of oligo-mesotrophic to mesotrophic waters should occur in the stand water: at the optimum, only 0–20 μg/l NO_3-N, 10–30 μg/l NH_4-N and 5–15 μg/l PO_4-P (Adamec, 1999a; Adamec and Lev, 1999; Cross et al., 2016). Nutrient concentrations ten times higher than this are well tolerated by AV and may contribute to its more rapid growth (Kamiński, 1987a; Adamec and Kovářová, 2006; Cross et al., 2015), but they simultaneously support the growth of phytoplankton or filamentous or blue-green algae and/or dense macrophyte stands.

10) A certain concentration of humic acids (or tannins) must be present in the water. The optimum may be either 5–10 mg C/l or 6–25 mg/l humic acids + tannins (Kamiński, 1987a, 1987b; Adamec, 1999a; Adamec and Lev, 1999; Adamec and Kovářová, 2006). AV can also grow well in slightly dystrophic waters in sand-pits (Adamec and Kučerová, 2013b; Cross et al., 2016) which indicates that it can grow well within a very broad concentration range of humic acids + tannins of ca. 2–40 mg/l. This range occurs in the vast majority of standing waters but higher concentrations (i.e., dark-brown water) are clearly unfavourable.

11) Based on many natural or artificial sites and outdoor cultures, AV can grow within a very broad pH range of ca. 5.0–9.0, but a much narrower range of ca. 5.7–7.6 was found under optimal growth conditions (Kamiński, 1987a; Adamec, 1997a, 1997b, 1999a; Adamec and Lev, 1999; Adamec and Kovářová, 2006; Cross et al., 2015, 2016). As pH is related to total alkalinity (i.e., HCO_3^- concentration) and CO_2 concentration, it is possible to conclude that the pH value itself is not primarily important but CO_2 concentration is. In line with this, total alkalinity itself is not important for AV growth as AV can grow in a very broad range from 0 to 5 meq/l (Adamec, 1995). Similarly, the very broad amplitude of total alkalinity reflects a very broad range of electrical conductivity at AV sites from 2 to 90 mS/m (Cross, 2012; Cross et al., 2016; L. Adamec, unpubl. data).

12) To enhance the potential spreading of AV by water birds, every single AV site should be surrounded by a chain of potential sites separated by only a few km. This requirement should contribute to the survival and spread of the population in a given area as its occurrence at a single site is often limited in time (Berta, 1961; Walters, 1979).

13) It follows from the above ecological requirements that stenotopic AV is relatively tolerant of many ecological factors. In conclusion, the suitability of AV habitats does not depend simply on one or two specific factors, but on the maintenance of a well-balanced combination of many ecological requirements (Adamec, 1999a; Cross, 2012). As a result of the combination of ecological factors, even a relatively small change in one factor (water chemistry, water level decline, density of dominant helophyte stand, etc.) can result in a dramatic change in AV population size or even extinction. On the other hand, new suitable microhabitats can also arise repeatedly.

3.5. Ecophysiological characteristics and carnivorous traits

As an aquatic carnivorous plant, the growth of AV is markedly supported by carnivory. In four outdoor or greenhouse growth experiments, feeding plants on prey (zooplankton) in 8–27 day experiments led to a marked and statistically significant increase of shoot length, number of adult leaf whorls, dry weight (DW) of plants, apical shoot growth rate, branching and RGR compared to unfed plants (Kamiński, 1987b; Adamec, 2000, 2008a; Adamec et al., 2010). In all experiments, the effect of prey feeding was strongest on branching: fed variants produced on average 0.42–1.39 branches per plant, while the unfed variants did not branch at all. The markedly enhanced growth parameters exhibited by shortening the doubling time of biomass in the fed variant from 10.6 d to only 6.4 d (Adamec et al., 2010).

AV is a typical aquatic photosynthetic C3 plant and a strict CO_2 user (Adamec, 1997b, 2008a; Adamec and Kovářová, 2006). Its CO_2 compensation point is within 4.6–11.4 μM in field-grown Polish plants (Adamec and Kovářová, 2006) and, similarly, within 5.0–7.2 μM in cultured plants (Adamec, 1997b, 2008a). These values lie within a common range reported for many strict CO_2 users among submerged plants (cf. Maberly and Spence, 1983). Feeding on prey did not influence the CO_2 compensation point though chlorophyll *a* content was slightly decreased (2.39 *vs.* 2.64 g/kg$_{DW}$) but photosynthetic rate increased significantly by 59% Adamec, 2008a). Under optimal conditions (22 °C, irradiance 120 W/m^2 of PAR), the CO_2 half-saturation constant (K_m) was 165 ± 77 μM and the maximum oxygen-based photosynthetic rate (as calculated from the light and CO_2 photosynthetic curves) was 78.0 and 98.8 mmol kg$_{FW}^{-1}$ h^{-1} (Adamec, 1997b). A similar value of 93.7 mmol kg$_{FW}^{-1}$ h^{-1} was also estimated in sprouting AV turions (Adamec, 2011b). AV thus exhibits very high photosynthetic rates in its shoots, comparable with the highest rates found in submerged plants (Adamec, 2011a, 2018a) which are a prerequisite for its rapid growth. Photosynthetic rate increased in a logarithmic way between 15 and 34 °C. Sun-adapted AV plants from outdoor culture were found to be markedly photophilous: their light compensation point was

6.0 W/m^2 of PAR (≈ 25 μmol quanta m^2 s^{-1}) and the photosynthetic rate was not light-saturated at 200 W/m^{-2} of PAR, either. The photosynthetic rate was the highest in the six youngest whorls of mature leaves and gradually declined in older shoot segments and the photosynthetic rate of isolated traps was only 67% of that of the intact shoot segments (Adamec, 1997b). The aerobic dark respiration rate of AV shoots and sprouting turions at 20 °C was within 5.2-9.2 mmol kg$_{FW}^{-1}$ h^{-1} and in shoots, it increased linearly from 15 to 34 °C (Adamec, 1997b, 2008a, 2011b). Feeding on prey did not increase the dark respiration rate significantly. A surprisingly high amount of starch and free sugars (totally 13.7% of DW) is lost in senescent shoot segments (Adamec, 2000).

Adult AV shoots exhibit a marked polarity of tissue N, P and Ca contents (Adamec, 2000). The N and P nutrient contents are the highest in shoot apices and decline steeply towards the old, senescent shoot segments, which indicates an effective reutilization (recycling) of N (60–90%) and P (61–67%) in basal senescent shoot segments (Adamec, 2000, 2016). However, the shoot Ca content exhibits a distinctly opposing polarity, with its maximum in the oldest segments. The shoot K content is nearly the same in shoot segments of different age or slightly higher in older ones, which indicates a zero or very low K reutilization from the senescent shoots, even at a very low (ca. 4 μM) ambient K^+ concentration (Adamec, 2000, 2016). AV plants thus continuously lose all Ca and (nearly) all K and Mg in senescent shoots and the lost cations must be replaced by an uptake of these from the ambient water or from prey (Adamec, 2018a). The high NH_4^+:NO_3^- ratio found in typical dystrophic habitats of AV reflects a marked uptake preference of NH_4^+ to NO_3^- from equimolar solutions (Adamec, 2000, 2016). The uptake of NH_4^+ or phosphate by apical and basal parts of the shoot exhibited about the same rate (Adamec, 2000), but the K^+ uptake by the apical parts was ca. 3.4 times faster (Adamec, 2016). However, the effect of prey feeding during precultivation on the subsequent nutrient uptake is ambiguous: prey feeding decreased the NH_4^+ uptake rate by 42%, but increased that of phosphate by 85% (Adamec, 2016). In a growth experiment, according to a simple model, AV plants fed on ostracods could cover ca. 73% of the increased amount of N, 49% of P and even > 100% of K and Mg in the increased biomass (*minus* the unfed controls) from prey (Adamec et al., 2010). This might indicate that prey capture in AV leads to a marked stimulation of K^+ and Mg^{2+} uptake by shoots from the ambient water which is similar to prey capture stimulating root nutrient uptake in terrestrial carnivorous plants (see Adamec, 2011a, 2018a).

Plumbagin is a yellow naphthoquinone occurring typically in all three genera of the Droseraceae and widespread within the eight families of the order Nepenthales (Juniper et al., 1989). Adamec et al. (2006) determined the presence of plumbagin in 14 world AV populations (Europe, Australia, Japan) but its topoisomer, 7-methyljuglone, was not detected. Using two analytical methods, the plumbagin content in apical shoot segments (shoot apex + 1st-6th leaf whorls) ranged between 1.2–6.0% DW and that in basal ones (7th-12th leaf whorls) between 0.4–3.9%; the content in apical segments was always 1.2–4 times higher than in basal ones. The mean content in turions was about 3.0% DW. The mean plumbagin content of ca. 2–3% DW in AV shoots corresponds to that found in two *Drosera* species (Kováčik and Repčák, 2006) or in *Dionaea muscipula* (Tokunaga et al., 2004). As no difference in plumbagin content was found between sun- and deep shade-adapted AV plants, only a marginal role of plumbagin in photoprotection may be assumed (Adamec et al., 2006). This supports the view that the main role of plumbagin in Droseraceae is as an antifeedant and antimicrobial, which protects AV plants against herbivores and microbial parasites (Tokunaga et al., 2004).

4. Genetic data

AV of several world populations was found to have 48 (2*n*) chromosomes (Hoshi et al., 2006, 2017; Cross, 2012). The chromosomes are

small in size, ca. 1 μm large and without any clear centromere position (Hoshi et al., 2017). The latter authors estimated the 1C genome size of two AV populations (Hungary, SW Australia) to be 0.62 pg and/or 602–607 Mbp. Great effort has been made to estimate the genetic variability among various world populations and specify their relatedness and possible origin. Generally, all studies using different methods reported an apparent genetic paradox that even very distant populations (e.g., Poland, Japan, Australia) are all genetically reminiscent of a monoclone although their biological traits (turion formation and its timing, shape of capsules, anthocyanin production, growth patterns) may clearly differ (Adamec and Tichý, 1997; Maldonado San Martín et al., 2003; Hoshi et al., 2006; Elansary et al., 2010; Cross, 2012). Maldonado San Martín et al. (2003) used random amplified polymorphic DNA (RAPD) to study the genetic variation on 12 accessions of AV from Europe, Australia and Japan. The low level of variation was confirmed in that only 21 of 151 RAPD primers gave polymorphic banding patterns. Analyses distinguished three clusters of closely-related plants: an Australian and Japanese accession, a Ukrainian, Russian and Romanian accession, and an accession from Poland and Germany. However, plant contamination by epiphytic cyanobacteria could explain the surprising variability between two tested German populations of the same origin (Cross, 2012). On the basis of comparing the 5.8S and internal transcribed spacer (ITS) in the 45S ribosomal DNA in an Australian and five temperate AV accessions, Hoshi et al. (2006) confirmed homologous nucleotide sequences within all tested populations with the ITS region (normally a very variable genetic marker sequence) being very conservative. A more recent sequencing study on six chloroplast intergenic regions and two mitochondrial gene flanking regions revealed only two different chloroplast haplotypes among all 15 AV accessions from four continents, generally separating European and non-European accessions, with two exceptions (Elansary et al., 2010). The genetic variation found in world AV populations was even lower than in other submerged plant species with a world-wide distribution. One may also speculate that an AV population within a site is genetically homogeneous and represents a monoclone, but this has never been verified.

In conclusion, at least one recent bottleneck followed by long-distance dispersal by water birds (as recent gene flow) combined with the founder effect, low mutation rates and dominant asexual reproduction could be responsible for the observed genetic uniformity (Maldonado San Martín et al., 2003; Hoshi et al., 2006; Elansary et al., 2010; Cross, 2012). Moreover, as all temperate and also some (sub)tropical Australian AV populations form dormant turions it seems probable that the ancestral AV population originated in the northern temperate zone, survived the glaciation periods in warmer refugia in deltas of big rivers (Danube, Dnieper, Volha) and, secondarily, was transferred by water birds to southern (sub)tropical zones in Africa and Australia (Cross, 2012).

Fourteen intraspecific hybrids between various AV populations have been prepared by crossing since 2014 (Y. Katagiri, unpubl. data). By growing these hybrids outdoors for several seasons, it was observed that their coloration is usually intermediate of that of the parents: if one of the parents has red coloration (i.e., Australian or African plants), the hybrid is at least reddish (Y. Katagiri and L. Adamec, unpubl. data). Similarly, if one of the parents belongs to a temperate population, the hybrid always forms turions in cold water in autumn and the same may apply also for the crossing between two Australian populations if one forms turions. Thus, anthocyanin synthesis and turion formation in AV have a dominant character. The hybrids are fully fertile and normally set seeds.

5. Threats and conservation

As already described in the above sections, AV is generally considered a stenotopic species though it may be rather tolerant of many individual ecological factors. However, the essence of suitable and optimal sites consists of a well-balanced combination of many ecological requirements (Adamec, 1999a; Cross, 2012) as its disturbance can lead to population decline or extinction. Over the last ca. 150 years, nearly 90% of all historically known AV sites have vanished worldwide (Cross, 2012). Based on literature data, the reasons for AV extinction can be freely subdivided into two groups (Walters, 1979; Adamec, 1995, 2005a; Shiyan and Andrienko, 2011; Cross, 2012; Kamiński, 2014; Jovanovska et al., 2017). The first group of reasons are evident and anthropogenic: direct destruction of a site, drainage and drying out, direct eutrophication of sites from adjacent agriculture, fishery or municipal pollution; other reasons may be recreation, motor boats, and more general land-use changes such as intensive agriculture and afforestation. Besides these obvious reasons, high concentrations of NO_3^-, NH_4^+ and SO_4^{2-} in acid precipitates can invisibly speed up habitat eutrophication. The second group of reasons is more or less independent of man and also occurs in the most protected nature reserves without any human impact: spontaneous habitat succession and water level fluctuations leading to water level decline. As the largest majority of AV sites (historical and extant) worldwide undergo frequent water level decline, global climate change can markedly speed up this decline and drying out. Evidently, very low water level (0–10 cm) is the most common and dangerous ecological threat at many AV sites and poses the common denominator which interconnects all consequences of site eutrophication, whatever the reason. The common scenario for these changes may be as follows (Adamec and Lev, 1999; Adamec, 2005a; Cross, 2012): after eutrophication of an AV site, an originally loose helophyte stand becomes taller and denser, and beside other unfavourable ecological changes (shading, cooling), it produces more slowly decomposable plant litter and the free water column depth decreases to zero. Thus, the naturally unavoidable process of shallow aquatic habitat succession is amplified and accelerated by the combination of eutrophication and water level decline; the next step is infilling by terrestrial helophyte stands followed finally by afforestation by wetland trees. In deeper eutrophicated sites, AV can be outcompeted by dense stands of submerged or rooted floating-leaved plants or filamentous algae (Cross et al., 2015).

As recently listed by Cross (2012), AV has been declared as Critically Endangered and officially protected in all European countries of its natural spread since 1982 (Threatened Plants Unit, 1982), though it may be extinct in the wild in some (e.g., Germany), and the same conservation status applies for Australia and Japan. It is also included in Annex I of the Bern Convention as a species requiring specific habitat conservation measures. Within the IUCN, it has recently been officially declared as Endangered B2ab (iii,v; The IUCN Red List of Threatened Species, 2017): "Area of occupancy estimated to be less than 500 km^2; severely fragmented or known to exist at no more than five localities; continuing decline, observed, inferred or projected, in any of the following: area, extent and/or quality of habitats or number of mature individuals". Due to the lack of information on its recent world distribution, AV has not been listed in the CITES appendices. AV of introduced populations is listed in the Red List of Plants in Switzerland as Endangered. In the Czech Republic, the natural AV population is declared as Extinct but allochthonous introduced populations as Endangered (Adamec and Kučerová, 2013b). The introduced Dutch AV population does not possess any conservation status. In Uganda, AV has a status of an Endangered species (Kalema et al., 2016).

In Europe, natural extant AV sites usually occur in protected areas – nature reserves or even national parks – without any direct, visible human impact (Kamiński, 2006, 2014; Shiyan and Andrienko, 2011; Jovanovska et al., 2017). However, this does not help much to protect the sites and the populations as the main sources or reasons of endangering come both from the watershed, the precipitates and also due to water level fluctuations and decline. Obviously, in the pre-industrial period, a relatively great turnover of AV sites occurred when the extinction of AV sites was associated with a continual rise of new sites within the same river basin or lake district (see Kamiński, 2006, 2014;

Shiyan and Andrienko, 2011). In Europe during the last ca. 150 years, the number of potentially suitable sites for AV has diminished rapidly but only a few of them have arisen in the last decades. Moreover, the extant natural spread in Europe is so fragmentary and the sites scattered across such long distances that natural spread is almost excluded. On the other hand, several successful introductions of AV plants from allochtonous populations to quite new regions have been conducted since 1908, mainly in the last 25 years (see above: Switzerland, Poland, Czech Republic, The Netherlands, Japan, USA; Koch, 1950; Adamec, 2005a; Kamiński, 2006, 2014; Cross, 2012; Adamec and Kučerová, 2013b; Lamont et al., 2013). Thus, it appears that purposeful introductions of AV plants from the last extant European populations to carefully selected potentially suitable sites in Europe (and other continents, see Lamont et al., 2013) might become an effective way to rescue the endangered world AV population (for the discussion of the risks see Adamec, 2005a). Successful introductions to the USA have indicated that the AV population alone is not able to endanger any local aquatic plant species (Lamont et al., 2013). Throughout Holocene history, distant AV populations were frequently naturally spread in this way by water birds over Europe (e.g., Berta, 1961).

6. Inspirations for further research

To gain further insight into AV biology, the following questions should be considered. One cluster of questions relates to weak generative reproduction. To understand why seed-set in AV is very limited in temperate populations, it is necessary to know what the natural longevity of pollen in open flowers is. It is generally accepted that high temperatures are the key factor for both AV flowering and fruiting (Adamec and Tichý, 1997; Cross et al., 2016). Yet what is the role of other trophic factors (high photosynthetic rate, carnivory) for flowering and fruiting? One out of the main ecological functions of seeds is to provide a long-distance transfer to new sites by water birds. What is the resistance of AV seeds in the digestion tracks for different groups of water birds? The first data on this show that it is very low (Cross et al., 2016). The estimated very low genetic variability between world AV populations is also attributed to dominant vegetative propagation. However, what could be the genetic variability within an AV population reproducing sexually? Over a dozen AV hybrids between different world populations have recently been prepared (Y. Katagiri, unpubl.). How are these hybrids able to be utilised for genetic studies? When have AV populations colonised Africa and Australia presumably from ancestral Eurasian sites?

Another cluster of questions relates to AV nutrition. It is known that some threshold concentration of organic substances is essential for AV growth (Kamiński, 1987b). How and why do humic acids and tannins at optimal concentrations optimise AV growth and development? Does carnivory in AV stimulate nutrient uptake from the ambient water by shoots like in terrestrial carnivorous plants (Adamec, 2011a, 2018a; Adamec et al., 2010)? What is the seasonal (daily) nutrient gain (consumption) for N, P and K from prey under natural conditions in field-grown AV?

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References

Adamec, L., 1995. Ecological requirements and the European distribution of the aquatic carnivorous plant *Aldrovanda vesiculosa* L. Folia Geobot. Phytotax 30, 53–61.

Adamec, L., 1997a. How to grow *Aldrovanda vesiculosa* outdoors. Carniv. Plant Newslett. 26, 85–88.

Adamec, L., 1997b. Photosynthetic characteristics of the aquatic carnivorous plant Aldrovanda vesiculosa. Aquat. Bot. 59, 297–306.

Adamec, L., 1999a. Seasonal growth dynamics and overwintering of the aquatic carnivorous plant *Aldrovanda vesiculosa* at experimental field sites. Folia Geobot. 34, 287–297.

Adamec, L., 1999b. Turion overwintering of aquatic carnivorous plants. Carniv. Plant Newslett. 28, 19–24.

Adamec, L., 1999c. Further notes on flowering and seed set of Aldrovanda vesiculosa. Flytrap News (Sydney) 12 (4), 9–12.

Adamec, L., 2000. Rootless aquatic plant *Aldrovanda vesiculosa*: physiological polarity, mineral nutrition, and importance of carnivory. Biol. Plant. 43, 113–119.

Adamec, L., 2003. Ecophysiological characterization of dormancy states in turions of the aquatic carnivorous plant Aldrovanda vesiculosa. Biol. Plant. 47, 395–402.

Adamec, L., 2005a. Ten years after the introduction of *Aldrovanda vesiculosa* to the Czech Republic. Acta Bot. Gall. 152, 239–245.

Adamec, L., 2005b. Quoi de nouveau du côté de la recherche sur Aldrovanda? Dionée 58, 22–28.

Adamec, L., 2008a. The influence of prey capture on photosynthetic rate in two aquatic carnivorous plant species. Aquat. Bot. 89, 66–70.

Adamec, L., 2008b. Respiration of turions and winter apices in aquatic carnivorous plants. Biologia 63, 515–520.

Adamec, L., 2008c. Survival of dried turions of aquatic carnivorous plants. Carniv. Plant Newslett. 37, 52–56.

Adamec, L., 2010. Tissue mineral nutrient content in turions of aquatic plants: does it represent a storage function? Fundam. Appl. Limnol. 176, 145–151.

Adamec, L., 2011a. Ecophysiological look at plant carnivory: why are plants carnivorous? In: In: Seckbach, J., Dubinski, Z. (Eds.), All Flesh is Grass. Plant-Animal Interrelationships. Cellular Origin, Life in Extreme Habitats and Astrobiology Vol. 16. Springer Science + Business Media B. V., Dordrecht - Heidelberg – London – New York, pp. 455–489.

Adamec, L., 2011b. Dark respiration and photosynthesis of dormant and sprouting turions of aquatic plants. Fundam. Appl. Limnol. 179, 151–158.

Adamec, L., 2016. Mineral nutrition in aquatic carnivorous plants: effect of carnivory, nutrient reutilization and K$^+$ uptake. Fundam. Appl. Limnol. 188, 41–49.

Adamec, L., 2018a. Ecophysiology of aquatic carnivorous plants. In: Ellison, A.M., Adamec, L. (Eds.), Carnivorous Plants: Physiology, Ecology, and Evolution. Oxford University Press, Oxford, U.K, pp. 256–269.

Adamec, L., 2018b. Germination and longevity of seeds of *Aldrovanda vesiculosa* and Utricularia vulgaris. Carniv. Plant Newslett. 47, 64–69.

Adamec, L., 2018c. Ecophysiological characteristics of turions of aquatic plants: a review. Aquat. Bot. 148, 64–77.

Adamec, L., Kovářová, M., 2006. Field growth characteristics of two aquatic carnivorous plants, *Aldrovanda vesiculosa* and Utricularia australis. Folia Geobot. 41, 395–406.

Adamec, L., Kučerová, A., 2013a. Overwintering temperatures affect freezing temperatures of turions of aquatic plants. Flora 208, 497–501.

Adamec, L., Kučerová, A., 2013b. [Rescue introductions of endangered species of aquatic plants to the protected landscape area Třeboňsko during 1994–2012.] In Czech. Issue South. Bohem. Mus. České Budějovice, Nat. Sci. 53, 59–69.

Adamec, L., Lev, J., 1999. The introduction of the aquatic carnivorous plant *Aldrovanda vesiculosa* to new potential sites in the Czech Republic: a five-year investigation. Folia Geobot. 34, 299–305.

Adamec, L., Tichý, M., 1997. Flowering of *Aldrovanda vesiculosa* in outdoor culture in the Czech Republic and isozyme variability of its European populations. Carniv. Plant Newslett. 26, 99–103.

Adamec, L., Gastinel, L., Schlauer, J., 2006. Plumbagin content in *Aldrovanda vesiculosa* shoots. Carniv. Plant Newslett. 35, 52–55.

Adamec, L., Sirová, D., Vrba, J., 2010. Contrasting growth effects of prey capture in two carnivorous plant species. Fundam. Appl. Limnol. 176, 153–160.

Afanas'ev, K.S., 1953. [New site of *Aldrovanda vesiculosa* L. in USSR] In. Russ. Bot. Zh. 38, 432–434.

Akeret, B., 1993. Ein neuer Fundort von *Aldrovanda vesiculosa* L. in der Nordschweiz und einige Bemerkungen zu *Stratiotes aloides* L. Bot. Helv. 103, 193–199.

Berta, J., 1961. Beitrag zur Ökologie und Verbreitung von *Aldrovanda vesiculosa* L.. Biologia 16, 561–573.

Creuwels, J., Pieterse, S., 2018. Checklist Dutch Species Register. Naturalis Biodiversity Centerhttps://doi.org/10.15468/rjdpzy. Checklist Dataset.

Cross, A., 2012. *Aldrovanda*. The Waterwheel Plant. Redfern Natural History Productions, Poole, Dorset, England.

Cross, A.T., 2013. Turion development is an ecological trait in all populations of the aquatic carnivorous plant Aldrovanda vesiculosa. Carniv. Plant Newslett. 42, 1–3.

Cross, A.T., Skates, L.M., Adamec, L., Hammond, C.M., Sheridan, P.M., Dixon, K.W., 2015. Population ecology of the endangered aquatic carnivorous macrophyte *Aldrovanda vesiculosa* at a naturalised site in North America. Freshwater Biol. 60, 1772–1783.

Cross, A.T., Adamec, L., Turner, S.R., Dixon, K.W., Merritt, D.J., 2016. Seed reproductive biology of the rare aquatic carnivorous plant *Aldrovanda vesiculosa* (Droseraceae). Bot. J. Linn. Soc. 180, 515–529.

Cross, A.T., Davis, A.R., Fleischmann, A., Horner, J.D., Jürgens, A., Merritt, D.J., Murza, G.L., Turner, S.R., 2018. Reproductive biology and prey-pollinator conflicts. In: Ellison, A.M., Adamec, L. (Eds.), Carnivorous Plants: Physiology, Ecology, and

Evolution. Oxford University Press, Oxford, U.K, pp. 294–313.

Darnowski, D.W., Bauer, U., Méndez, M., Horner, J.D., Płachno, B.J., 2018. Prey selection and specialization by carnivorous plants. In: Ellison, A.M., Adamec, L. (Eds.), Carnivorous Plants: Physiology, Ecology, and Evolution. Oxford University Press, Oxford, U.K, pp. 285–293.

Degreef, J.D., 1986. Aldrovanda vesiculosa. Publication of the French Carnivorous Plant Society, Liège.

Doronina, A.J., 2014. [On new records of Carex livida (Wahlenb.) Willd. and Aldrovanda vesiculosa L. in Leningrad Province.]. In Russ. Byull. Mosk. Obshch. Ispyt. Prir. Otd. Biol. 119, 62.

Elansary, H.O.M., Adamec, L., Štorchová, H., 2010. Uniformity of organellar DNA in Aldrovanda vesiculosa, an endangered aquatic carnivorous species, distributed across four continents. Aquat. Bot. 92, 214–220.

Fleischmann, A., Cross, A.T., Gibson, R., Gonella, P.M., Dixon, K.W., 2018a. Systematics and evolution of Droseraceae. In: Ellison, A.M., Adamec, L. (Eds.), Carnivorous Plants: Physiology, Ecology, and Evolution. Oxford University Press, Oxford, U.K, pp. 45–57.

Fleischmann, A., Schlauer, J., Smith, S.A., Givnish, T.J., 2018b. Evolution of carnivory in angiosperms. In: Ellison, A.M., Adamec, L. (Eds.), Carnivorous Plants: Physiology, Ecology, and Evolution. Oxford University Press, Oxford, U.K, pp. 22–41.

Gorissen, I., 2015. Flora der Region Bonn (Stadt Bonn und Rhein-Sieg-Kreis). Decheniana – Beih. (Bonn) 40, 361.

Grime, J.P., 1979. Plant Strategies and Vegetation Processes. Wiley, Chichester.

Hoshi, Y., Shirakawa, J., Hasebe, M., 2006. Nucleotide sequence variation was unexpectedly low in an endangered species, Aldrovanda vesiculosa L. (Droseraceae). Chromos. Bot. 1, 27–32.

Hoshi, Y., Azumatani, M., Suyama, C., Adamec, L., 2017. Determination of ploidy level and nuclear DNA content in the Droseraceae by flow cytometry. Cytologia 82, 321–327.

Huber, H., 1961. Aldrovanda L. In: Hegi, G. (Ed.), Illustrierte Flora von Mitteleuropa. Carl Hanser Verlag, München, pp. 18–20 ed. 2.4 (2).

Jovanovska, D., Mladenova, M., Minova, E., Melovska, N., Melovski, L., 2017. Rediscovery of Aldrovanda vesiculosa L. and new data on its distribution in the Republic of Macedonia. Acta Musei Maced. Sci. Nat. 20, 31–40.

Juniper, B.E., Robins, R.J., Joel, D.M., 1989. The Carnivorous Plants. Academic Press, London.

Kalema, J., Namaganda, M., Bbosa, G., Ogwal-Okeng, J., 2016. Diversity and status of carnivorous plants in Uganda: towards identification of sites most critical for their conservation. Biodivers. Conserv. 25, 2035–2053.

Kamiński, R., 1987a. Studies on the ecology of Aldrovanda vesiculosa L. I. Ecological differentiation of A. vesiculosa population under the influence of chemical factors in the habitat. Ekol. Pol. 35, 559–590.

Kamiński, R., 1987b. Studies on the ecology of Aldrovanda vesiculosa L. II. Organic substances, physical and biotic factors and the growth and development of A. vesiculosa. Ekol. Pol. 35, 591–609.

Kamiński, R., 2006. [Restitution of Waterwheel plant (Aldrovanda vesiculosa L.) in Poland and recognition of factors deciding on its survival in temperate climate.]. In Polish. Prace Ogr. Bot. Uniw. Wrocł. 8, 105.

Kamiński, R., 2014. Aldrovanda vesiculosa L. Polska czerwona księga roślin. Paprotniky i rośliny kwiatowe, 3rd edition. Polska Akademia Nauk, Cracow, Poland, pp. 237–239.

Koch, W., 1950. Zum Vorkommen der Aldrovanda vesiculosa L. in der Schweiz. Ber.

Schweiz. Bot. Ges. 60, 324–325.

Kováčik, J., Repčák, M., 2006. Naphthoquinones content of some sundews (Drosera L.). Carniv. Plant Newslett. 35, 49–51.

Lamont, E.E., Sivertsen, R., Doyle, C., Adamec, L., 2013. Extant populations of Aldrovanda vesiculosa (Droseraceae) in the New World. J. Torrey Bot. Soc. 140, 517–522.

Lloyd, F.E., 1942. The Carnivorous Plants. Chronica Botanica, Waltham, MA, USA.

Maberly, S.C., Spence, D.H.N., 1983. Photosynthetic inorganic carbon use by freshwater plants. J. Ecol. 71, 705–724.

Maldonado San Martín, A.P., Adamec, L., Suda, J., Mes, T.H.M., Štorchová, H., 2003. Genetic variation within the endangered species Aldrovanda vesiculosa (Droseraceae) as revealed by RAPD analysis. Aquat. Bot. 75, 159–172.

Muravnik, L.J., Vasil'ev, A.J., Potapova, J.J., 1995. Ultrastructural aspects of digestive gland functioning in Aldrovanda vesiculosa. Russ. J. Plant Physiol. 42, 1–8.

Okada, H., 2008. Pollination system of Aldrovanda vesiculosa (Droseraceae), a critically endangered aquatic plant in Japan. Makinoa New Ser. (Kochi) 7, 93–100.

Płachno, B.J., Muravnik, L.E., 2018. Functional anatomy of carnivorous traps. In: Ellison, A.M., Adamec, L. (Eds.), Carnivorous Plants: Physiology, Ecology, and Evolution. Oxford University Press, Oxford, U.K, pp. 167–179.

Płachno, B.J., Adamec, L., Kozieradzka-Kiszkurno, M., Świątek, P., Kamińska, I., 2014. Cytochemical and ultrastructural aspects of aquatic carnivorous plant turions. Protoplasma 251, 1449–1454.

Poppinga, S., Bauer, U., Speck, T., Volkov, A.G., 2018. Motile traps. In: Ellison, A.M., Adamec, L. (Eds.), Carnivorous Plants: Physiology, Ecology, and Evolution. Oxford University Press, Oxford, U.K, pp. 180–193.

Shiyan, N.M., Andrienko, T.L., 2011. [Aldrovanda vesiculosa L. in the Prypiat river basin]. Ukr. Ukr. Bot. Zh. 68, 517–525.

Šimura, J., Spíchal, L., Adamec, L., Pěnčík, A., Rolčík, J., Novák, O., Strnad, M., 2016. Cytokinin, auxin and physiological polarity in the aquatic carnivorous plants Aldrovanda vesiculosa and Utricularia australis. Ann. Bot. 117, 1037–1044.

The IUCN, 2017. Red List of Threatened Species. (Accessed 30 April 2012). www.iucnredlist.org/details/162346/0.

Thiele, K.R., Coffey, S.C., 2014. The taxonomic status of Aldrovanda vesiculosa var. rubescens (Droseraceae). Nuytsia 24, 19–20.

Threatened Plants Unit, 1982. List of Rare, Threatened and Endemic Plants in Europe. Nature and Environment ser. No. 27. Council of Europe, Strasbourg, France.

Tokunaga, T., Dohmura, A., Takada, N., Ueda, M., 2004. Cytotoxic antifeedant from Dionaea muscipula Ellis: a defensive mechanism of carnivorous plants against predators. Bull. Chem. Soc. Jap. 77, 537–541.

Vilkonis, K.K., 2003. [Aldrovanda vesiculosa (Droseraceae) in Lithuania.]. Russ. Bot. Zh. 88, 56–58.

Walters, S.M., 1979. Conservation of the European flora: Aldrovanda vesiculosa L., a documented case-history of a threatened species. In: Hedberg, I. (Ed.), Systematic Botany, Plant Utilization and Biosphere Conservation. Almqvist and Wiksell International, Stockholm, pp. 76–82.

Weber, H.E., 1995. Aldrovanda. In: Hegi, G. (Ed.), Illustrierte Flora von Mitteleuropa, Band IV, Teil 2A, 3. Auflage. Blackwell Wissenschafts, Berlin, pp. 34–37.

Westermeier, A.S., Sachse, R., Poppinga, S., Vögele, P., Adamec, L., Speck, T., Bischoff, M., 2018. How the carnivorous waterwheel plant (Aldrovanda vesiculosa) snaps. Proc. R. Soc. B-Biol. Sci. 285, e20180012. www.plantarium.ru/page/image/id/542035.html.