RESEARCH ARTICLE

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Endozoochory of aquatic ferns and angiosperms by mallards in Central Europe

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Abstract

1. Modern literature on plant dispersal by birds focuses mainly on the importance of frugivory and scatter-hoarding, yet recent studies show that endozoochory by migratory waterbirds is an important mechanism of long-distance dispersal for a broad range of plants. Nevertheless, there is a lack of empirical field studies that identify the plants dispersed by waterbirds in a comprehensive manner. In particular, so far there are no detailed studies of the level of spatial variation in the plant taxa dispersed by a waterbird vector, and no clear demonstration that aquatic ferns can be dispersed by endozoochory. Consequently, we remain ignorant of the networks of dispersal interactions between granivorous waterbirds and plants.

2. Five sets of faecal samples (total *n* = 215) were collected from mallards *Anas platyrhynchos* on autumn migration in the Hevesi-holm and Balaton regions in Hungary, central Europe. Intact diaspores were extracted, identified and their germinability assessed under standard conditions. The plant communities recorded at different sites were compared with PERMANOVA and other multivariate methods.

3. Macrospores of the floating watermoss *Salvinia natans* were recorded in 32 samples, and a total of 16 macrospores germinated, providing the first field demonstration of endozoochory of ferns by birds. Of 21 angiosperm taxa recorded (of which eight germinated), 13 were terrestrial species, although the most abundant taxa were aquatic species such as the alkali bulrush *Bolboschoenus maritimus* and the sago pondweed *Potamogeton pectinatus*. Two naturalized alien species, the common fig *Ficus carica* and the hackberry *Celtis occidentalis* were also recorded. Only four of the taxa had an endozoochory syndrome. The plant taxa dispersed varied at two different spatial scales, with minor but significant differences between samples from sites separated by less than 1 km, and major differences between the two regions separated by *c.* 220 km.

4. *Synthesis*. This is a unique study of the spatial variation in plants dispersed by endozoochory by a migratory waterfowl species, with a high taxonomic resolution and the first demonstration of avian endozoochory of ferns. We recorded eight taxa not previously reported as dispersed by mallards, showing how more empirical studies are essential, so we can understand which plants are dispersed by migratory birds. We found evidence that networks of interactions between granivorous waterbird vectors and dispersed plants vary spatially.

1 | INTRODUCTION

Research to date on avian dispersal of plants has focused mainly on frugivory or on scatter-hoarding (Forget et al., 2011; Pesendorfer, Sillett, Koenig, & Morrison, 2016; Vander Wall & Moore, 2016). However, both classic and more recent research has shown that migratory waterfowl can disperse a broad range of other plants by endozoochory (De Vlaming & Proctor, 1968; Green, Soons, Brochet, & Kleyheeg, 2016; van Leeuwen, van der Velde, van Groenendael, & Klaassen, 2012). These include angiosperms whose diaspores lack any obvious morphological adaptations for long-distance dispersal (Costea et al., 2016; Soons, Brochet, Kleyheeg, & Green, 2016). Nevertheless, only a handful of studies have considered which plants are actually dispersed in the field via gut passage by widespread waterfowl species such as mallards *Anas platyrhynchos* (e.g. Charalambidou & Santamaría, 2005; Green, Frisch, Michot, Allain, & Barrow, 2013; Wilkinson, Lovas-Kiss, Callaghan, & Green, 2017). Since morphological syndromes do not allow us to predict a priori which plants are dispersed (Soons et al., 2016), there is an urgent need for extensive field studies, so that we can begin to characterize the networks of dispersal interactions between waterbirds and plants.

Even the broad taxonomic diversity of plants dispersed by waterfowl is currently unclear (Green et al., 2016). It has only recently been demonstrated that waterfowl disperse viable bryophyte fragments by endozoochory (Wilkinson et al., 2017). Despite long-standing speculation about the potential of aquatic ferns to disperse via waterbirds (Reynolds, Miranda, & Cumming, 2015; Ridley, 1930), this has never been conclusively demonstrated. Laboratory experiments support the potential for epizoochory of *Azolla* over short distances (Coughlan, Kelly, & Jansen, 2017), and the potential for endozoochory of *Marsilea* (Malone & Proctor, 1965).

Mallards are important plant vectors both during seasonal, migratory movements (Viana, Santamaría, Michot, & Figuerola, 2013) and during daily movements within a landscape (Kleyheeg, Treep, De Jager, Nolet, & Soons, 2017). However, no previous studies have considered in detail what spatial differences there are between different waterbodies in the plants that mallards or other waterbirds disperse by endozoochory (i.e. equivalent to studies comparing frugivore-plant dispersal interactions in nearby forest patches; Bascompte & Jordano, 2007). In this study, our aim is to quantify and compare endozoochory of aquatic and terrestrial plants by mallards on autumn migration at different wetlands of Hungary, central Europe. We identify diaspores deposited in faeces with a high taxonomic resolution, assess the germinability of diaspores and analyse the extent of spatial variation in the dispersal of different plant taxa. We also provide the first demonstration of endozoochory of aquatic ferns. We consider how many of the taxa recorded were not previously known to be dispersed by ducks, and the implications of our results for long-distance dispersal processes.

2 | MATERIALS AND METHODS

2.1 | Study area and sample processing

Sampling was carried out in two different regions of Hungary, the Hevesi-holm and the riviera of Lake Balaton (Figure 1). Five sets of faecal samples were collected in the autumn of 2016 with a combined total of 215 samples. In the Balaton region, we collected 90 samples on the 20th (*n* = 52) and 23rd of October (*n* = 38) from two adjacent sites on the northern shore of Lake Balaton (46.9141°N, 17.89270°E; 46.91736°N, 17.8929°E), and 25 samples on 21st October at the Tihany Inner-Lake (46.90783°N, 17.88707°E). In the Hevesi-holm region, we collected 50 samples on 20th September from Lake Tisza (47.643938°N, 20.660793°E), and 50 samples from the Tisza River (47.60477°N, 20.71102°E; Figure 1). All the locations are situated within protected areas, Natura 2000 sites and BirdLife Important Bird Areas.

Lake Balaton is the largest (596 km^2) freshwater lake in central Europe, but has a mean depth of only 3.2 m (Istvánovics et al., 2007). It is *c*. 18,000 years old (Cserny & Nagy-Bodor, 2000), and its trophic state has changed from meso- to eutrophic over the last millennium (Korponai et al., 2011). The northern shore is covered with reed (*Phragmites australis*) and *Typha* beds, with extensive submerged macrophytes. The nearby Tihany Inner-Lake (0.2 km^2) is a highly eutrophic volcanic lake covered with reed (*Ph. australis*) and other emergent plants.

Lake Tisza is a reservoir which is usually drained in the late autumn then filled again in the spring with water from the Tisza River. It covers 127 km^2 with average depth varying from 0.7 and 2.5 m in the shallow bays. Our samples were collected from one of the shallowest bays, which is largely covered by reed (*Ph. australis*) and is rich in submerged and emergent macrophytes (Király, Molnár, Bölöni, Csiky, & Vojtkó, 2008). The lake is connected to the Tisza River by several channels. The banks of the Tisza River are largely covered by willow-groves and poplar plantations invaded by the false indigo bush (*Amorpha fruticosa*; Király et al., 2008). During our sampling visits, some mallards were seen feeding within floating mats of the floating watermoss, *Salvinia natans*, at the Lake Tisza and Tisza River sites.

Monospecific flocks of resting mallards likely to be on migration (see Section 4) were located on the shoreline of the study sites. Fresh faecal material was then collected early in the morning from the points where birds were resting, immediately after they were flushed by our
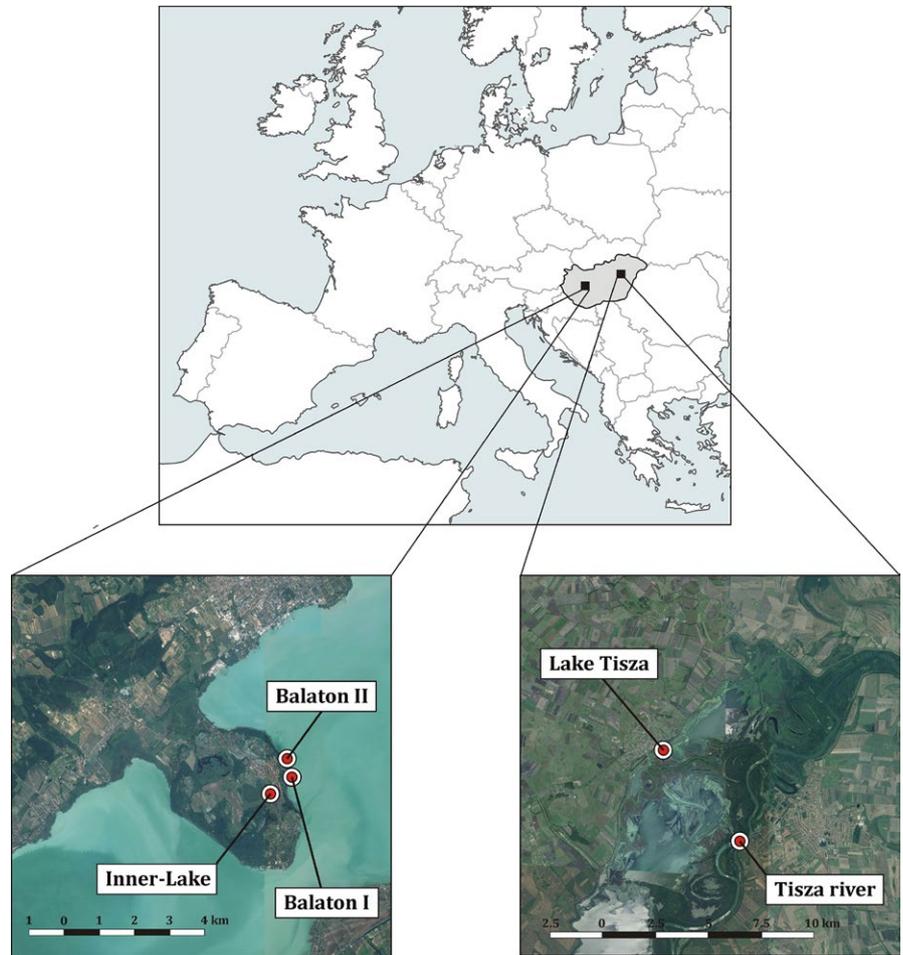
FIGURE 1 Location of the study areas in Hungary (lower images taken from Google Earth)

approach, leaving at least one metre distance between samples to minimize the risk of sampling the same individual repeatedly. The faecal samples were immediately examined, removing any contamination from soil or vegetation from the sample using tweezers, then placed in ziplock plastic bags. The samples were then kept in the fridge (4°C) until subsequent laboratory investigations, which were completed within 6 months. Faecal samples were weighed on a balance (Ohaus EP213C Explorer® Pro), then washed on a 100 μm sieve using deionized water before inspection under a binocular microscope. Intact diaspores, for which no cracks or missing parts were detectable, were collected and quantified. Angiosperm seeds were photographed and identified based on their shape, size, colour and seed coat pattern following recent literature (Bojnanský & Fargašová, 2007; Cappers, Bekker, & Jans, 2012). Fern macrospores were identified following Weijdema, Brinkkemper, Peeters, and Geel (2011). Dispersal syndromes for each taxon were taken from the plant trait database Baseflore (Julve, 1998),

Diaspores were then placed in an Eppendorf® tube filled with deionized water until germination trials. Germinability tests were conducted on Petri-dishes filled with nutrient-free Agar gel for terrestrial plants, and in tissue plates filled with deionized water for aquatic plants (Araceae, Haloragaceae, Potamogetonaceae, Salviniaceae). These were then placed in germination chambers set at 12 hr of light at 24°C, and 12 hr of darkness at 18°C. Germination tests were run for 54 days.

2.2 | Statistical analysis

To compare the number of intact diaspores of a given taxon in the five sets of samples, we used a Kruskall–Wallis H test. This test was only performed for those taxa with diaspores recorded in a combined total of at least six faecal samples. Where significant differences were detected, we used Dunn's test (Dinno, 2017) to identify which sites differed significantly.

Several additional analyses were conducted using functions implemented in the R package "vegan." In order to analyse differences among plant community compositions recorded at the five sites, we used nonparametric permutational ANOVA (i.e. PERMANOVA; Anderson, 2001) using the *adonis* function. The latter analysis was based on a Bray–Curtis dissmiliarity matrix, composed of values between 0 and 1 reflecting the degree of dissimilarity among the five sites. Using these analyses, we inspected the differences in community compositional centroids among sites, but further analyses were performed to inspect differences in group homogeneities, using the function *betadisper* (Anderson, 2006). Using the latter procedure, we calculated the degree of dispersion of each sample from the compositional centroid at each sampling site. This procedure is often referred to as a test for difference in β diversity among the sampling sites. Tukey's HSD tests were used to determine which sites differed in β diversity. Additionally, a posteriori analysis was performed, using

the function *simper*, to identify community members that significantly contributed to among-site differences. To establish statistical significance, the empirical *p* value of each variable was calculated using 1,000 random permutations. Rarefaction analysis showed that our results were not strongly influenced by the differences in number of samples between sites (Figure S1).

Graphical presentation of the among-sites differences was conducted using principal coordinates analyses, performed using the *cmdscale* function on the above mentioned Bray–Curtis dissimilarity matrix. All statistical analyses were conducted in R statistical software package (version 3.3.2; R Core Team, 2017). Adonis, betadisper and simper functions were implemented in R package vegan (version 2.4-3., Oksanen et al., 2017), while Tukey's HSD and cmdscale were implemented in R package stats (version 3.4.0, R Core Team, 2017). These analyses were run after excluding samples that contained no diaspores.

3 | RESULTS

The mean mass of fresh faecal samples was 3.721 ± 0.185 g (*M* ± *SE*). A total of 1,327 intact diaspores were found from the 215 samples, belonging to 22 plant species, including 21 angiosperms and an aquatic fern (381 macrospores of *S. natans*, Table 1). These taxa represent 15 families (Table 1), with five families of aquatic plants (including three submerged, two floating and four emergent taxa), and 10 families of terrestrial plants (13 taxa). Only four of these 22 taxa are recognized as having an "endozoochory syndrome" (Table 2).

Almost half the diaspores (47%) were retrieved from the two sets of samples from Lake Balaton, which included 484 seeds of common fig (*Ficus carica*, naturalized alien, Uotila, 2011b; Table 1). Fig trees are very abundant around Lake Balaton and were present at the sampling sites (Király et al., 2008). The hackberry *Celtis occidentalis*, a park tree from North America that is a widespread naturalized alien in Hungary (Bartha et al., 2015; Uotila, 2011a), was represented by one seed. All other plant taxa recorded were native to Hungary. Most plant taxa recorded were rare, with seven taxa represented by only one seed, and another four taxa recorded only in a single sample (Table 1). Overall, 33% of faecal samples contained at least one diaspore (Table 1).

Propagules of three plant species were recorded in more than five faecal samples: the alkali bulrush *Bolboschoenus maritimus*, the sago pondweed *Potamogeton pectinatus* and *S. natans* (Table 1). Diaspore numbers of these three taxa differed significantly among the sampling sites (*B. maritimus* χ^2 = 38.33, *df* = 4, *p* < .0001; *P. pectinatus* χ^2 = 10.59, *p* = .032; *S. natans* χ^2 = 47.27, *p* < .0001). For *B. maritimus*, significantly more diaspores were recorded at the Tihany Inner-Lake and at Balaton II than at the other three sampling sites (Table 1). Significantly more *P. pectinatus* seeds were recorded from the Tihany Inner-Lake than at the Lake Tisza and at Balaton II sampling sites. We only found *S. natans* macrospores at the two neighbouring sites in the floodplain of the Tisza river, with significantly more diaspores than in the other sites (Table 1).

According to PERMANOVA, the species compositional centroids differed significantly among sampling sites (*df* = 1, *F* = 18.381, *p* < .0001, Figure 2). The *simper* post hoc test indicated that *S. natans*, *B. maritimus*, *F. carica* and *Cyperus fuscus* were the taxa that made the highest contribution to the among-sites dissimilarities. The β-diversity of the propagules found in the samples differed significantly among the sampling sites, according to the results of betadisper (*df* = 4, *F* = 4.31, *p* = .0036). Tukey's HSD test showed significant β-diversity difference (*p* = .0034) between the Balaton II and Lake Tisza sites.

Out of the 22 species of plants recorded in the faecal samples, diaspores from nine taxa germinated. Overall, 4% of all diaspores germinated. Three taxa had particularly high viability (*B. maritimus* 25%, *Eleocharis palustris* 31% and *Ranunculus sceleratus* 25%, Table 2). Sixteen *S. natans* macrospores germinated (4% of the total, Figure 3). All taxa with over 20 diaspores detected in the faecal samples contained viable seeds (as shown by the germination tests), with the notable exception of *F. carica* represented by over 400 seeds in the samples (Table 2).

4 | DISCUSSION

We have demonstrated endozoochory for aquatic ferns and a range of angiosperm taxa by migratory waterfowl in central Europe, and the germinability of all abundant taxa apart from cultivated figs. Although we do not know the distance involved, the diaspores were undoubtedly moved by the mallards between ingestion at feeding sites and egestion at the roost sites sampled (see Kleyheeg et al., 2017 for typical examples of daily movements for mallards not on migration). In terms of the number of faecal samples processed and the diversity and level of identification of intact diaspores recorded, this study represents the most detailed field study of endozoochory by mallards to date (see Green et al., 2016 for review). It is also the first to make a detailed comparison of the spatial variation in plants dispersed. Our results illustrate the importance of mallards as a vector of dispersal for widely distributed (e.g. *Polygonum aviculare*, *Cyperus fuscus*, Bartha et al., 2015) and rare (*Myriophyllum verticillatum*, Bartha et al., 2015) plant species, as well as naturalized aliens (*C. occidentalis*, Uotila, 2011a). Another naturalized alien *F. carica* (Uotila, 2011b) had the highest number of seeds in our study, but none germinated because this cultivar produces their fruits parthenocarpically and these have non-viable seeds in Hungary. *Bolboschoenus maritimus* and *P. pectinatus* were the angiosperms recorded in the highest number of samples. Seeds of both these subcosmopolitan species have often been recorded in the diet of ducks (Soons et al., 2016) and have been used as models for experimental studies of endozoochory (Brochet, Guillemain, Gauthier-Clerc, Fritz, & Green, 2010; Espinar, Garcia, Figuerola, Green, & Clemente, 2004; Santamaría et al., 2003). Modelling based on banding recoveries and feeding experiments suggests that, during migrations, mallards can disperse seeds of these taxa and of *Schoenoplectus lacustris* (which we also recorded) over extraordinary distances with maxima exceeding 400 km (Viana et al., 2013).

We sampled mallards during the autumn migration period when birds move through Hungary towards wintering grounds in the eastern

TABLE 1 Total number of diaspores (TD), number of samples with diaspores (NS), and maximum number of diaspores in any given sample (Max) for plants recorded in five sets of mallard faecal samples. For three taxa recorded in ≥6 samples, no shared superscript letters in the TD column indicate a statistically significant difference between the sites

Family	Species	Tisza River (n = 50) TD	NS	Max	Lake Tisza (n = 50) TD	NS	Max	Balaton I. (n = 52) TD	NS	Max	Balaton II. (n = 38) TD	NS	Max	Inner-Lake (n = 25) TD	NS	Max	Total TD	NS
Salviniaceae	Salvinia natans[1]	253[a]	14	96	128[a]	18	39	–[b]	–	–	–[b]	–	–	–[b]	–	–	381	32
Adoxaceae	Sambucus nigra	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1	1	1
Araceae	Lemna minor[2]	–	–	–	15	1	15	–	–	–	–	–	–	–	–	–	15	1
Betulaceae	Betula pendula	–	–	–	–	–	–	–	–	–	2	1	2	–	–	–	2	1
	Carpinus betulus[3]	–	–	–	–	–	–	–	–	–	1	1	1	–	–	–	1	1
Cannabaceae	Celtis occidentalis[1,4]	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1	1	1
Cyperaceae	Bolboschoenus maritimus	–[a]	–	–	1[a]	1	1	–[a]	–	–	45[b]	7	18	15[b]	8	5	61	16
	Cyperus fuscus[1]	–	–	–	–	–	–	–	–	–	20	3	16	166	1	166	186	4
	Eleocharis palustris	–	–	–	13	1	13	–	–	–	–	–	–	–	–	–	13	1
	Schoenoplectus lacustris	–	–	–	1	1	1	–	–	–	53	2	33	–	–	–	54	3
	Schoenoplectus tabernaemontani[1]	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1	1	1
Fabaceae	Trifolium pratense	–	–	–	1	1	1	–	–	–	–	–	–	–	–	–	1	1
Haloragaceae	Myriophyllum verticillatum[3]	14	2	13	42	2	37	–	–	–	–	–	–	–	–	–	56	4
Moraceae	Ficus carica[5]	–	–	–	–	–	–	117	1	117	367	1	367	–	–	–	484	2
Plantaginaceae	Plantago major	–	–	–	–	–	–	–	–	–	–	–	–	9	1	9	9	1
Poaceae	Digitaria sanguinalis	–	–	–	–	–	–	1	1	1	–	–	–	–	–	–	1	1
	Echinochloa crus-galli	–	–	–	–	–	–	5	1	5	–	–	–	9	3	7	14	4
Polygonaceae	Polygonum aviculare	–	–	–	–	–	–	3	1	3	–	–	–	2	2	1	5	3
Potamogetonaceae	Potamogeton pectinatus	15[ab]	2	11	–[a]	–	–	1[ab]	1	1	–[a]	–	–	4[b]	3	2	20	6
	Potamogeton pusillus	6	3	2	–	–	–	–	–	–	–	–	–	–	–	–	6	3
Ranunculaceae	Ranunculus sceleratus	–	–	–	1	1	1	–	–	–	13	2	11	–	–	–	14	3
Solanaceae	Solanum dulcamara[3]	1	1	1	–	–	–	–	–	–	–	–	–	–	–	–	1	1
	Total	289	22	96	202	20	39	127	3	117	501	14	369	208	12	176	1,327	71

[1] Not reported in Anas spp. diet by Soons et al. (2016).
[2] Unidentified seeds from the same genus were reported in Anas spp. diet by Soons et al. (2016).
[3] Not reported in mallard diet by Soons et al. (2016).
[4] Alien taxa.
[5] Ancient cultivated species.

Plants				Samples (n = 215)	
Family	Species	Dispersal syndrome[a]	Length (mm)[b]	TD	NG
Salviniaceae	Salvinia natans	Hydrochory	0.6	381	16
Adoxaceae	Sambucus nigra	Endozoochory	4	1	0
Araceae	Lemna minor	Hydrochory	0.8	15	0
Betulaceae	Betula pendula	Anemochory	3.2	2	0
	Carpinus betulus	Anemochory	5.77	1	0
Cannabaceae	Celtis occidentalis	Endozoochory	5.61	1	0
Cyperaceae	Bolboschoenus maritimus	Anemochory	3.78	61	18
	Cyperus fuscus	Barochory	0.9	186	4
	Eleocharis palustris	Epizoochory	1.87	13	4
	Schoenoplectus lacustris	Hydrochory	3.2	54	4
	Schoenoplectus tabernaemontani	Barochory	2.94	1	0
Fabaceae	Trifolium pratense	Epizoochory	1.9	1	0
Haloragaceae	Myriophyllum verticillatum	Hydrochory	1.95	56	4
Moraceae	Ficus carica	Endozoochory	1.66	484	0
Plantaginaceae	Plantago major	Barochory	1.58	9	0
Poaceae	Digitaria sanguinalis	Barochory	1.75	1	0
	Echinochloa crus-galli	Epizoochory	2.84	14	0
Polygonaceae	Polygonum aviculare	Barochory	1.9	5	1
Potamogetonaceae	Potamogeton pectinatus	Hydrochory	4.05	20	3
	Potamogeton pusillus	Hydrochory	2.25	6	0
Ranunculaceae	Ranunculus sceleratus	Hydrochory	0.84	14	3
Solanaceae	Solanum dulcamara	Endozoochory	2.28	1	0
	Total			1,327	57

[a]From the BASEFLOR database (Julve, 1998).
[b]From the LEDA database (Kleyer et al., 2008), the Digital seed atlas (Cappers et al., 2012) or from our own measurements.

Mediterranean and Black Sea regions (Wetlands International, 2017). Ringing recoveries connect mallards in Hungary with sites over 1,600 km away in Russia and France (Faragó, 2009). In the Lake Balaton area, mallard numbers increase during autumn migration from less than 500 individuals in early September to c. 2,700 individuals in October, before decreasing again to c. 1,000 in November (Bankovics, 1997). The entire Hungarian mallard population was estimated to contain around 40,000 individuals in October 2014 (Faragó, Jánoska, Hajas, & Kovács, 2016), and is part of the Eastern Europe/Black Sea & Eastern Mediterranean flyway population of c. 2 million birds (Wetlands International, 2017). Given the number of mallard individuals present in our study area, the plant taxa we recorded are likely to undergo regular long-distance dispersal events along migratory flyways via endozoochory (Viana, Santamaría, & Figuerola, 2016; Viana et al., 2013).

The proportion of samples containing at least one propagule was similar to that observed in previous studies of waterfowl (van Leeuwen et al., 2012). From our 22 taxa recorded, diaspores of six (27%) were not previously recorded in the diet of European dabbling ducks, and a further two species were not previously reported from mallards (Soons et al., 2016; table I). This illustrates how much remains to be learned about which plants are dispersed through endozoochory by migratory waterfowl. This is vital knowledge, as these vectors are particularly able to provide the long-distance dispersal required e.g. for redistribution in response to global change (Viana et al., 2016).
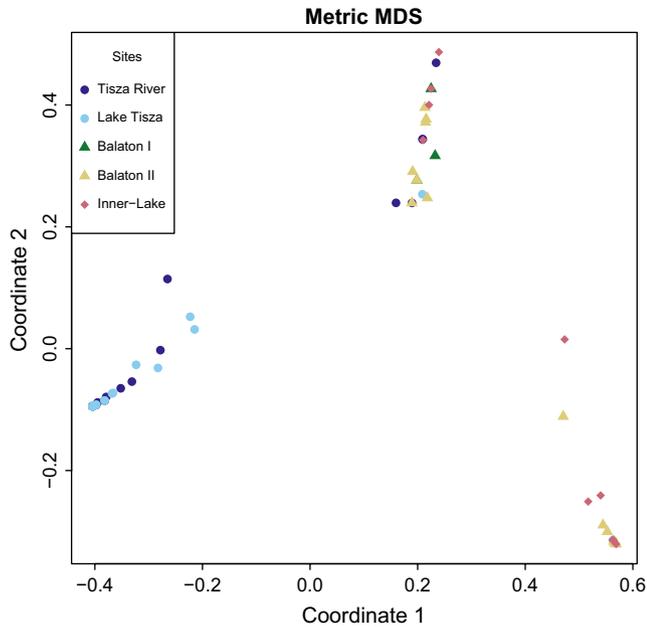
Metric MDS

FIGURE 2 Ordination based on a Principal Coordinates Analysis of plant communities among the five sample collection sites. The first two PCO axes are shown

In the plant trait database Baseflore (Julve, 1998), most of these 22 species were assigned to dispersal syndromes based on water, wind or self-dispersal (Table 2). These mechanisms provide much lower maximum dispersal distances than those provided by endozoochory (Bullock et al., 2017). It is notable how mallards disperse trees such as the Silver birch *Betula pendula* or the European hornbeam *Carpinus betulus* that are generally assumed to be wind-dispersed. We have seen mallards searching for and taking birch seeds as they are blown onto the water surface, and also feeding on them when they are concentrated at the shoreline by wave action (Figure 4). Only four plant species with a fleshy fruit were assigned to an endozoochory syndrome. The majority of taxa we recorded were terrestrial plants, supporting the findings of Soons et al. (2016) in a meta-analysis of the diet of European dabbling ducks. Nevertheless, diaspores of aquatic taxa were generally more abundant, and all three

taxa found in six or more samples were aquatic. Most (55%) of the plant taxa we recorded were only recorded in one or two samples. Thus, we would need to study many more samples to record all the plant taxa dispersed by mallards using our study sites (Figure S1), as indicated by rarefaction analyses of mallard gut contents at other European sites (Soons et al., 2016).

At the level of individual plant taxa, significant differences in diaspore numbers did not fully coincide with the spatial divide between samplings from the Hevesi-holm and those from the Lake Balaton region further west. We even found significant differences in the frequency of *B. maritimus* between two samplings at Lake Balaton separated by 373 m and by 3 days. Patchy distribution of seeds in the environment and individual variation in feeding behaviour or movements of ducks (e.g. Green, 1998; Kleyheeg et al., 2017) can translate into important variation in dispersal processes at a fine scale. There was only a clear geographical separation for *S. natans*, which was restricted to the Hevesi-holm (Table 1). *Salvinia natans* is mainly found around the big rivers in Hungary, and is absent from the Lake Balaton area (Bartha et al., 2015). On the other hand, when the whole community of plant diaspores was analysed, there was a clear separation between the eastern and western sample sets (Figure 2), which is likely to reflect important differences in the plant communities present in the two study regions. However, sampling in the Lake Balaton and Hevesi-holm regions was conducted a month apart, so the spatial differences observed may have been confounded with this temporal change.

Our finding of viable macrospores of the pteridophyte *S. natans* is very important, as this represents the first demonstration of avian endozoochory of viable fern diaspores in the wild. Most fern diaspores are dispersed by wind, and a smaller fraction by water (Sharpe, Mehltreter, & Walker, 2010). A few previous studies have demonstrated endozoochorous dispersal of ferns by other animals, including slugs and insects (Boch, Berlinger, Prati, & Fischer, 2016; Boch et al., 2013). Fern spores have been shown experimentally to remain viable after passage through the gut of large bats (Sugita, Ootsuki, Fujita, Murakami, & Ueda, 2013). Dispersal of viable spores by endozoochory has also been demonstrated in the field for mice (Arosa, Ramos, Quintanilla, & Brown, 2010) and reindeer (Bråthen et al., 2007).

We have demonstrated the viability of fern diaspores after avian gut passage for one fern species, but there is evidence to suggest that

FIGURE 3 Photos of *Salvinia natans* female gametophytes extracted from mallard faeces (a) before germination and (b) after germination, showing the archegonia (dark brown dots). The vertical bars represent 1 mm. Photographed by Á. Lovas-Kiss
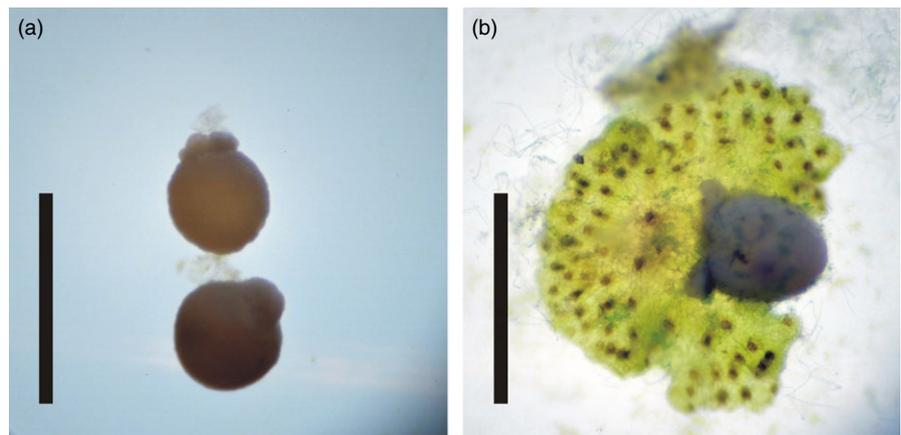
FIGURE 4 Silver birch *Betula pendula* seeds concentrated on a lake shoreline where mallards were feeding. Photographed by A. J. Green

birds are also vectors for other aquatic and terrestrial ferns. Extinct and extant birds have sometimes been reported to ingest fern sporangia or spores (Arosa et al., 2009; James & Burney, 1997; Sharpe et al., 2010). Malone and Proctor (1965) did experimental feeding trails with mallards and four other waterbird species using the hairy waterclover *Marsilea vestita*, and recorded passage of intact sporocarps among all bird species. A *Marsilea* sporocarp was recorded from the gizzard of a killdeer (*Charadrius vociferus*) collected in Texas (De Vlaming & Proctor, 1968). It has often been suggested that waterbirds can disperse the alien fern *Azolla filiculoides* (Reynolds & Cumming, 2015), and there is experimental support for epizoochory over distances of a few metres (Coughlan et al., 2017). *Azolla filiculoides* megasporocarps were found in faecal samples of swans and coots in the field, but the viability of the spores was not established (Green, Jenkins, Bell, Morris, & Kingsford, 2008). Dispersal of ferns as nest material by hummingbirds has also been reported (Osorio-Zuniga, Fonturbel, & Rydin, 2014).

Salvinia natans is a widespread Eurasiatic taxon, with the major populations in central and eastern Europe and Asia, extending to Southern Europe and Northern Africa (Allen, 2011). It is an alien species in Cuba and Jamaica (Allen, 2011), and the states of New York and Massachusetts (https://plants.usda.gov/). Ridley (1930) considered *S. natans* to be too large to be dispersed by birds via epizoochory, but speculated that "as the species often occur in isolated pools, it is probable that their spores are somehow transported by birds." Over 80 years later, we have confirmed his suspicion.

In central Europe, *S. natans* produces male microspores and female macrospores within sporocarps from late August to late September, with the release of sporocarps into the water beginning in late September (Gałka & Szmeja, 2013). When not eaten by birds, the sporocarps eventually sink to the bottom of the water column, and during winter the sporocarp walls disintegrate within the sediments. In April, the spores rise up to the water surface and begin to form gametophytes. In late April, the female gametophyte develops archegonia and the male gametophyte develops the antheridia. After fertilization occurs, the zygote forms and produces the sporophyte (Gałka & Szmeja, 2013). When placed for germination, the macrospores we extracted from faeces initially sank to the bottom of our tissue plates, but most floated to the surface after germination. Our laboratory conditions are likely to have accelerated their germination. After surviving gut passage in nature, it is possible that the macrospores delay germination until spring when day length and temperatures increase. When plants germinate sooner because of gut passage, there can be benefits from reduced intraspecific competition, or costs from increased exposure to herbivory (Figuerola & Green, 2004). In our faecal samples, we found only female macrospores, which produced gametophytes with archegonia (Figure 3). We recorded none of the male microspores that *S. natans* also produces, probably because the microspores are less than 20 μm in diameter (Lawalrée, 1964) and passed through our sieve. It is likely that microspores are also dispersed by mallards when they ingest sporocarps. We found no intact sporocarps in the faeces, and they are likely to have been broken within the gizzard.

In conclusion, this study illustrates how much our understanding of plant dispersal can be advanced by focused research on migratory waterfowl as vectors. We can now construct effective spatial models on the dispersal of plants by waterfowl (Kleyheeg et al., 2017; Viana et al., 2016), but we still have very limited information as to which plants are dispersed by which waterbird species. Endozoochory in nature goes well beyond the "endozoochory syndrome," so we cannot simply rely on syndromes if we wish to predict how plants can disperse and these mechanisms relate to plant distributions. Our study provides a unique insight into the networks of dispersal interactions between waterbirds and plants, and the extent of spatial variation that exists in these networks. However, it focuses on the mallard as a particularly abundant and widespread vector, and future work must compare the plants dispersed by other duck and waterbird species in an equally comprehensive manner, so that plant-vector networks can be fully characterized, and we can begin to understand the extent of functional redundancy between vectors.

AUTHORS' CONTRIBUTIONS

Á.L.-K. and A.J.G. conceived the ideas and designed methodology; Á.L.-K. and B.V. collected the data; Á.L.-K. and O.V. analysed the data; Á.L.-K., A.M.V. and A.J.G. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

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