**Brief Communication**

**Waterfowl endozoochory: An overlooked long-distance dispersal mode for *Cuscuta* (dodder)**

Mihai Costea\(^1\), Saša Stefanović\(^2\), Miguel A. García\(^3\), Susan De La Cruz\(^4\), Michael L. Casazza\(^5\), and Andy J. Green\(^6\)

**Premise of the Study:** Dispersal of parasitic *Cuscuta* species (dodders) worldwide has been assumed to be largely anthropomorphic because their seeds do not match any previously known dispersal syndrome and no natural dispersal vectors have been reliably documented. However, the genus has a subcosmopolitan distribution and recent phylogeographic results have indicated that at least 18 historical cases of long-distance dispersal (LDD) have occurred during its evolution. The objective of this study is to report the first LDD biological vector for *Cuscuta* seeds.

**Methods:** Twelve northern pintails (*Anas acuta*) were collected from Suisun Marsh, California and the contents of their lowest part of the large intestine (rectum) were extracted and analyzed. Seed identification was done both morphologically and using a molecular approach. Extracted seeds were tested for germination and compared to seeds not subjected to gut passage to determine the extent of structural changes caused to the seed coat by passing through the digestive tract.

**Key Results:** Four hundred and twenty dodger seeds were found in the rectum of four northern pintails. From these, 411 seeds were identified as *Cuscuta campestris* and nine as most likely *C. pacifica*. The germination rate of *C. campestris* seeds after gut passage was 55%. Structural changes caused by the gut passage in both species were similar to those caused by an acid scarification.

**Conclusions:** Endozoochory by waterbirds may explain the historical LDD cases in the evolution of *Cuscuta*. This also suggests that current border quarantine measures may be insufficient to stopping spreading of dodger pests along migratory flyways.

**Keywords:** *Anas acuta*; Convolvulaceae; identification; invasive plants; long-distance dispersal; northern pintail; parasitic plants; pest; seed viability

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“The dispersal of the Dodders to remote islands is very puzzling.”

Ridley, 1930

Seeds of the parasitic plant genus *Cuscuta* (dodder) have been considered “unspecialized” or “non-adapted” because they lack a morphological dispersal syndrome (Ridley, 1930; Kuijt, 1969; Dawson et al., 1994; Costea and Tardif, 2006). Natural dispersal by wind (Lyshede, 1984) and water have been anecdotally suggested for a select number of species (reviewed by Dawson et al., 1994; Costea and Tardif, 2006), but there is no evidence to suggest that these vectors allow long-distance dispersal (LDD). No other natural dispersal vectors have been recognized for *Cuscuta* seeds. For example, the evolution of subg. *Grammica*, the largest infrageneric group of *Cuscuta* (ca. 150 sp.) distributed mostly in the Americas, most likely involved a transoceanic dispersal from South Africa to South America (Stefanović et al., 2007; García et al., 2014). LDD occurred subsequently from the...
North or South American *Grammica* clades to some islands and virtually to all the other continents; e.g., *C. gymnocarpa* Engelm. and *C. acuta* Engelm. to the Galápagos Islands (Costea et al., 2015a); *C. sandwichiana* Choisy to Hawaii; *C. tasmanica* Engelm. and *C. victoriana* Yunck. to Australia (Costea et al., 2013); *C. kilimanjari* Oliv. to eastern Africa; *C. hyalina* Roth. to Africa and Asia (Costea and Stefanović, 2010); *C. chinensis* Lam. (var. *chinensis*; Costea et al., 2011) and *C. australis* R.Br. to Asia (see more cases in García et al., 2014).

Numerous *Cuscuta* species are major global pests, capable of drastically reducing the yield of numerous agricultural/horticultural crops or invading natural ecosystems (Dawson et al., 1994; Parker and Riches, 1993). The long-distance movement of such dodder species has long been thought to take place exclusively via contaminated seeds of various crops or Asian herbal products (Beal, 1910; Knepper et al., 1990; Dawson et al., 1994; Costea and Tardif, 2006). As a result, most countries have adopted legislation measures for surveillance and quarantine at the border to prevent the introduction of foreign *Cuscuta* spp. within their territory (Costea and Tardif, 2006). It is therefore important to know if *Cuscuta* seeds can also undergo LDD via nonhuman vectors.

The objective of this short-note is to report for the first time the endozoochory of *Cuscuta* (dodder, Convolvulaceae) seeds by a migratory waterfowl (northern pintails *Anas acuta* L.; Anatidae), and to discuss the implications of this finding.

**MATERIALS AND METHODS**

As part of a broader study of seed dispersal by waterfowl in the San Francisco Bay area, 11 northern pintails (hereafter referred to as “pintails”), were collected from Wings Landing, Suisun Marsh, San Francisco Bay area, and examined to identify the dodder species. Five of these seedlings were identified through DNA barcoding, using ITS (nuclear) and trnL-F (plastid) DNA sequences. Seedlings were frozen in liquid nitrogen and pulverized using solid glass beads (3 and 6 mm; Fisher Scientific, Waltham, Massachusetts, USA) and a mixer mill (MM 300, Retsch GmbH (Haan, Germany); 1 min at 30 Hz). DNA extractions, polymerase chain reaction (PCR) reagents and conditions, and amplicon purifications followed the protocols detailed in Stefanović et al. (2007). Cleaned PCR products were sequenced at the McGill University and Génome Québec Innovation Centre (Montreal, Quebec, Canada). A total of four ITS and six trnL-F sequences were analyzed and deposited in GenBank (accession numbers KU761496–KU761506). Sequences were aligned manually using Se-Al version 2.0a11 (Rambaut, 2002) and compared with our database containing a large number of *Cuscuta* species from the subgenus *Grammica* used in our previous broad-scale phylogenetic analyses of this group (Stefanović et al., 2007; Stefanović and Costea, 2008), as well as more recent analyses targeting specifically the *Cuscuta pentagona* Engelm./*campestris* species group (Costea et al., 2015a). To further characterize newly obtained DNA sequences, we compared them with those deposited in Genbank using BLAST. The remaining sixth seedling from NOPI 29 was grown using *Plectranthus scutellarioides* (L.) R.Br. (Lamiaceae) as a host in the University of Toronto, Mississauga greenhouse. At maturity, flowers of this dodder specimen were collected, dissected, and examined to identify the species morphologically. Vouchers of this plant were deposited in the TRTE and WLU herbaria as NOPI 29-7, 2 August 2015, *García s.n.* (herbarium voucher at TRTE and spirit specimen at WLU).

Morphology of all the seeds was initially surveyed with a Nikon SMZ1500 stereomicroscope. Thirty of the 320 remaining *Cuscuta* seeds were rehydrated and examined to determine the extent of morphological and structural changes caused by their passing through the digestive tract of pintails. Subsequently, seeds were cut in half through the hilar region, perpendicular to the hilum scar, subjected to a hexamethyldisilazane (HMDS) treatment as an alternative method to critical point drying (Wright et al., 2011), mounted on specimen stubs, and coated with 30 nm of gold using an Emitech K550 sputter coater (Quorum Technologies, Lewes, UK). Examination of the surface and seed coat structure was conducted with a Hitachi SU-1500 Scanning Electron Microscope (SEM) at 3 KV. Seeds were compared to those in a morphological database of *Cuscuta* seeds (Costea, unpublished data) after a search of the dodder species present at Suisun Marsh (Vasey et al., 2012; Consortium of California Herbaria, 2016).

After the identification of the seeds retrieved from the pintails (see Results below), typical dodder seeds of the same species that had not been subjected to gut passage were prepared and examined as indicated above from the following herbarium specimens: (1) *Cuscuta campestris*: USA, California, Sonoma Co., September 2007, Cadman et al. 2832 (WLU); (2) Riverside Co., 28 July 1994, Sanders 15174 (UCR); (3) San Bernardino Co., 1 September 2000, Provance 2227B (UCR); (4) *Cuscuta pacifica* Costea and M.A.R. Wright: USA, California, Humboldt Co., 28 August 1941, C.C. and S.K. Harris 1175 window on filter paper placed on top of a layer of cardboard soaked in distilled water inside petri dishes.

Another 20 seeds, similar morphologically to the ones used above, were kept dry and germinated on 15 May at the University of Toronto, Mississauga. The seven seedlings produced (six from NOPI 29 and one from a different bird labeled NOPI 30) were used to identify the dodder species. Five of these seedlings were identified through DNA barcoding, using ITS (nuclear) and trnL-F (plastid) DNA sequences. Seedlings were frozen in liquid nitrogen and pulverized using solid glass beads (3 and 6 mm; Fisher Scientific, Waltham, Massachusetts, USA) and a mixer mill (MM 300, Retsch GmbH (Haan, Germany); 1 min at 30 Hz). DNA extractions, polymerase chain reaction (PCR) reagents and conditions, and amplicon purifications followed the protocols detailed in Stefanović et al. (2007). Cleaned PCR products were sequenced at the McGill University and Génome Québec Innovation Centre (Montreal, Quebec, Canada). A total of four ITS and six trnL-F sequences were analyzed and deposited in GenBank (accession numbers KU761496–KU761506). Sequences were aligned manually using Se-Al version 2.0a11 (Rambaut, 2002) and compared with our database containing a large number of *Cuscuta* species from the subgenus *Grammica* used in our previous broad-scale phylogenetic analyses of this group (Stefanović et al., 2007; Stefanović and Costea, 2008), as well as more recent analyses targeting specifically the *Cuscuta pentagona* Engelm./*campestris* species group (Costea et al., 2015a). To further characterize newly obtained DNA sequences, we compared them with those deposited in Genbank using BLAST. The remaining sixth seedling from NOPI 29 was grown using *Plectranthus scutellarioides* (L.) R.Br. (Lamiaceae) as a host in the University of Toronto, Mississauga greenhouse. At maturity, flowers of this dodder specimen were collected, dissected, and examined to identify the species morphologically. Vouchers of this plant were deposited in the TRTE and WLU herbaria as NOPI 29–7, 2 August 2015, *García s.n.* (herbarium voucher at TRTE and spirit specimen at WLU).
FIGURE 1 Morphological and structural changes caused by the passing of *Cuscuta* seeds through the digestive tract of northern pintail. (A-C) Passed seeds of *C. campestris*; note that the external palisade layer persisted on irregular portions of the seed (A), in the hilum area (B), or it was completely eliminated. (D, E) Passed seeds of *C. pacifica*; the seed coat is fragmented and the external palisade layer was entirely removed. (F-H) Surface detail and anatomy of seed coat in passed seeds of *C. campestris*: (F) Hilum area (black arrows delineate the hilum); (G, H) Sclereids of the internal palisade layer were brought to the surface of the seed coat because the epidermis and external palisade layer were entirely digested. (I-K) Surface detail and structure
RESULTS

Of the 11 pintails collected from Suisun Marsh, four of them had intact *Cuscuta* seeds in the rectum. Two individuals had a single seed, one individual (NOPI 30) had six seeds, and the fourth individual (NOPI 29) had 412 seeds. The majority of *Cuscuta* seeds were small, 0.7–1.2 mm long. However, nine of the *Cuscuta* seeds examined from NOPI 29 were larger, 1.4–1.9 mm long, indicating that two different dodder species had been ingested by this individual.

Barcoding showed that the small dodder seeds found inside the rectum of pintails belong to *Cuscuta campestris* (field dodder). All sequences generated in this study (four ITS and six trnL-F) were either identical to or had ≤ 2bp differences compared with those of *C. campestris* obtained for our previous studies (Stefanović et al., 2007; Stefanović and Costea, 2008; Costea et al., 2015a). Also, the BLAST search of online DNA databases showed that the sequences from the seedlings are compatible with *C. campestris*, with the highest scores having 100% query coverage and 99–100% identity with *C. campestris* and other closely related members of the *C. campestris/pentagona* species group. The same species identity, *C. campestris*, was obtained through the morphological examination of dissected flowers produced by the mature *Cuscuta* plant grown in the greenhouse. The nine larger seeds found in NOPI 29 were identified morphologically as most likely belonging to *C. pacifica* (Pacific salt-marsh dodder).

All the 80 seeds tested for viability belonged to *Cuscuta campestris*: 23 seeds germinated by 9 February (28.75%), and 44 by 23 March (55%). Germination of the nine seeds of *C. pacifica* was not tested because of their insufficient number. The passage through the digestive tract of pintails modified significantly the structure of the seed coat in both *Cuscuta* species (Fig. 1). In *C. campestris*, the seed coat maintained its integrity, remaining attached to the endosperm (Fig. 1A-C); in *C. pacifica* the seed coat fragmented and detached from the endosperm (Fig. 1D, E). In both species, the epidermis with dome-like cells, which is always present in seeds not subjected to gut passage (Fig. 1J, P), was entirely stripped out. The external palisade layer, also characteristic to *Cuscuta* seeds (Fig. 1K, R), was entirely digested in *C. pacifica* (Figs. 1D, E, M, N), and partially or totally eliminated in *C. campestris* (Figs. 1A-C, G, H). Remnants of the external palisade layer may persist on irregular surfaces in *C. campestris* (Fig. 1A), but most often it can be found only in concave areas or in the hilar zone (Fig. 1B, F). Thus, in both dodder species, after the digestion process, the testa was reduced to the internal palisade layer, which was brought to the surface of seeds (Fig. 1G, H, M, N). Also, in both species, the hilum, which is responsible for inhibition in *Cuscuta*, becomes completely exposed, i.e., visible as a nearly invisible line in *C. campestris* (Fig. 1F; compare with 1I), and more conspicuous in *C. pacifica* (Fig. 1L; compare with 1O).

DISCUSSION

Zoochory has not previously been proposed as a dispersal mode for *Cuscuta*, but in retrospect the existing literature provides much support for our finding since *Cuscata* spp. seeds have already been reported in the diets of several species of migratory waterfowl (Cottam, 1939; Martin and Uhler, 1939; Chamberlain, 1959; Goodrick, 1979) and shore birds (Beltzer, 1991). However, none of these studies identified the *Cuscuta* species involved or tested the viability of passed seeds. In this study, the seeds of *C. campestris* retrieved after gut passage were viable and germinated at rates comparable to those reported for scarified seeds of this species at 21–24°C (e.g., Hutchison and Ashton, 1980; Benvenuti et al., 2005). Dormancy of *Cuscuta* seeds is physical, imposed by the impermeable seed coat with two palisade layers (e.g., Hutchison and Ashton, 1979; Lynshed, 1984; Jayasuriya et al., 2008). The structural changes reported after gut passage, especially the fragmentation of the seed coat in *C. pacifica*, are similar to those observed in *Cuscuta* after sulfuric acid scarification (Costea, unpublished data). Although we only found small numbers of seeds in three of the four ducks where *Cuscuta* was present, we only inspected a short section of the hindgut which holds a very small proportion of seeds carried through the entire digestive system (Brochet et al., 2010a).

Waterbirds provide a major ecosystem service by dispersing plants that lack a fleshy fruit and hence are not dispersed by frugivores (Green and Elmborg, 2014). The potential of migratory waterbirds to disperse plants over long distances and to oceanic islands has long been recognized (Darwin, 1859; Proctor, 1968; Carlquist, 1967). Experimental and field studies suggest that waterfowl are major but largely overlooked vectors for a broad range of wetland and terrestrial plants, including many species with “non-adapted” seeds like *Cuscuta* (Brochet et al., 2009, 2010b; Soons et al., 2016). Dodder seeds or fruits may be washed into wetlands by rainfall, making them available to dabbling ducks such as pintails. Modeling confirmed that dabbling ducks readily disperse seeds over hundreds of kilometers or more (Viana et al., 2013). Among other Convolvulaceae with subcosmopolitan distribution, Proctor (1968) showed experimentally that viable seeds of *Convolvulus arvensis* L. can be retained in the gut of shore birds for up to 144 h, which would be enough to cross the Pacific Ocean (Gill et al., 2009). Thus, as previously suggested for fleshy-fruited plants (e.g., Popp et al., 2011), our findings indicate that waterbirds or shore birds may explain the historical LDD events that took place in the evolution of *Cuscuta*.

Pintails wintering in California, Mexico, the Caribbean, and Central America undertake rapid long-distance migratory movements northwards to various parts of North America and Russia (Miller et al., 2005; Arzel et al., 2006). Similarly, pintails wintering in the Mediterranean Basin and Africa or in southeastern Asia (e.g., Japan) migrate to various northern areas of Europe and Asia (Arzel et al., 2006; Hupp et al., 2011). Although northern pintail migration routes are usually not transoceanic, vagrants occasionally
cross the Atlantic or Pacific (e.g., Flint et al., 2009). However, this particular duck species is probably not the dispersal vector involved in most historical LDD events that took place in the evolution of Cuscuta. One possible exception is C. sandwichiana, which is part of a North American clade but is endemic to Hawaii (Garcia et al., 2014; Costea et al., 2015b), where pintails winter regularly (e.g., Uvardy and Englis, 2001). Endozoochory by shore birds is the most likely explanation for other historical LDD events (Carlquist, 1967).

Cuscuta pacifica is the typical dodder of saline tidal marshes on the Pacific Coast (Costea et al., 2009), including at Suisun Marsh (Barbour, 2007; Vasey et al., 2012, referred to as “C. salina”). Cuscuta subinclusa Durand & Hilg., a closely related species (Costea et al., 2009), which is also present in the area (Vasey et al., 2012; Consortium of California Herbaria, 2016), has similar seeds morphologically (Costea et al., 2006) but it grows mostly on shrubs and trees. The seeds of all three Cuscuta species are enclosed in indescent fruits, which are usually persistent on the hosts in dense infructescences until the spring. For these reasons, it is more likely that the large seeds belong to C. pacifica which, like C. campestris, parasitize herbaceous hosts (Costea et al., 2009) and their fruits are more accessible to pintails feeding at ground level. Although this is not one of the LDD cases highlighted by García et al. (2014), the dispersal of C. pacifica over 2000 km of coast from British Columbia, Canada, via Washington, Oregon, and California in the USA, to Baja California in Mexico, may have involved pintails or other migratory waterbirds.

Cuscuta campestris is perhaps the most common weedy dodder worldwide (Costea et al., 2015a), and its ubiquitous presence has until now been considered to be explained solely as a result of human dispersal through contaminated seed crops. Our findings suggest that avian endozoochory may have also contributed to the widespread distribution of this species. For example, in this study the presence at Suisun Marsh shows that C. campestris movement is not necessarily linked to agricultural practices. Ducks have probably dispersed field dodder (and perhaps other species) within North America, Europe, and Asia. It has been recently reported (Costea et al., 2015a) that C. gymnocarpa, which is endemic to the Galapagos, is in fact a form of C. campestris that has evolved in the archipelago after a LDD event from the mainland. Thus, the possibility of endozoochory opens a new direction of research in the ecology and biogeography of Cuscuta. Finally, the potential for endozoochory reported here suggests that enforcement of the current border quarantine measures will not be sufficient to completely curtail the international movement of field dodder and other Cuscuta pest species.

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LITERATURE CITED

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