GENETIC IDENTIFICATION OF SUBMERSED DODDER (*CUSCUTA*: CONVOLVULACEAE) IN TEXAS, U.S.A.

Casey R. Williams

BIO-WEST, Inc.
1405 United Dr. Suite 111
San Marcos, Texas 78666, U.S.A.
cwilliams@bio-west.com

Nicholas P. Tippery

Department of Biological Sciences
University of Wisconsin-Whitewater
800 W Main St.
Whitewater, Wisconsin 53190, U.S.A.
tipperyn@uww.edu

Donald H. Les

Department of Ecology and Evolutionary Biology
University of Connecticut
75 N Eagleville Rd.
Storrs, Connecticut 06269, U.S.A.
donald.les@uconn.edu

ABSTRACT

Cuscuta plants that were observed growing entirely submersed and parasitizing the aquatic macrophyte *Hygrophila polysperma* in the San Marcos and Comal Rivers in Texas, are identified genetically as *C. obtusiflora*. This note documents the first molecular identification of any *Cuscuta* species naturally growing underwater or parasitizing a submersed aquatic plant.

RESUMEN

Las plantas de *Cuscuta* que se observaron creciendo completamente sumergidas y parasitando a la macrófita acuática *Hygrophila polysperma* en los ríos San Marcos y Comal en Texas, se identifican genéticamente como *C. obtusiflora*. Esta nota documenta el primer identificación molecular de cualquier especie de *Cuscuta* creciendo bajo el agua naturalmente o parasitando una planta acuática sumergida.

INTRODUCTION

Cuscuta L. is a cosmopolitan genus of approximately 200 species that are recognized as slender, twining, holoparasitic plants largely lacking chlorophyll (Godfrey & Wooten 1981; Diggs et al. 1999; Stefanović et al. 2007; Braukmann et al. 2013; García et al. 2014). As a result of their parasitic nature, members of the genus are often considered noxious terrestrial weeds of crops and other plants (Dawson et al. 1994; Lanini & Kogan 2005; Costea & Tardif 2006). Their reduced morphology often presents a barrier to effective identification, and molecular tools are becoming increasingly useful for discriminating species and reevaluating ecological interactions (Costea & Stefanović 2009; García et al. 2014; Costea et al. 2020).

Around 30 *Cuscuta* species grow in Texas, U.S.A, comprising mostly native species with sparse geographic distributions (Kartesz 2015) but also including some rather widespread natives (e.g., *C. gronovii* Willd. ex Schult., *C. pentagona* Engelm.) and two nonindigenous species (*C. japonica* Choisy, *C. suaveolens* Ser.) (POWO 2022). Individual *Cuscuta* species are known to parasitize anywhere from a few closely related species to a wide range of neighboring plant species (García et al. 2014; Costea et al. 2020). Both woody and herbaceous plants are capable of being parasitized, although herbaceous hosts are more common (Yuncker 1932). *Cuscuta* plants generally parasitize terrestrial plants, although interactions have been documented across a large number of obligate wetland plants (Les 2018, 2020).

An herbarium specimen collected in 1984 (*Ertter 5486*) documented an interaction between a *Cuscuta* plant and a submersed *Hygrophila polysperma* (Roxb.) T. Anderson in the San Marcos River, a spring-fed system located in Hays County, Texas. Annotation labels indicated a tentative identification of the *Cuscuta* plant as *C. obtusiflora* Kunth or *C. pentagona* but also noted the absence of flowers or fruits. In 2010, while conducting an aquatic plant survey of the San Marcos River, several patches of *Cuscuta* again were noted growing on

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submersed individuals of *H. polysperma* (Fig. 1). Further investigation confirmed the parasitic interaction of *Cuscuta* on submersed *H. polysperma* plants (Fig. 1). Subsequent visits to the original site and the nearby Comal River confirmed that *Cuscuta* persists on submersed *H. polysperma* host plants in both rivers throughout the year. The *Cuscuta* plants in the Comal River were robust and more localized than those in the San Marcos River. No flowers were observed on submersed *Cuscuta* specimens, but in early 2013 we observed flowering *Cuscuta* growing on emergent *H. polysperma* plants in the San Marcos River. The river was experiencing lower than average water depths due to drought. To obtain a more precise identification of the submersed *Cuscuta* species, material was collected from both the San Marcos and Comal rivers and analyzed using molecular methods.

MATERIALS AND METHODS

DNA was extracted, amplified, and sequenced for the nuclear internal transcribed spacer (ITS) region using the ITS5 and ITS4 primers (White et al. 1990) and for the plastid *trnL-trnF* spacer region using the 'c', 'd', 'e', and 'f' primers (Taberlet et al. 1991), following the methods reported by Tippery & Les (2011). Comparison sequences, selected to represent the diversity of the genus *Cuscuta*, were obtained from GenBank for ITS and *trnL-trnF* (McNeal et al. 2007; Stefanović et al. 2007; García et al. 2014; Costea et al. 2020). Sequence similarity was determined by a BLAST search (https://blast.ncbi.nlm.nih.gov/), confirmed with a manual sequence alignment for each gene.

RESULTS AND DISCUSSION

Sequence comparison revealed that the submersed *Cuscuta* specimens from both locations exactly matched sequences that were previously reported for *Cuscuta obtusiflora* var. *glandulosa* Engelm. ITS (GenBank accession number EF194675; Stefanović et al. 2007) and *trnL-trnF* (KT371736; Costea et al. 2015b). Newly obtained sequences were deposited in GenBank under accession numbers KJ812201 (ITS) and OL435813 (*trnL-trnF*) for the Comal River plants, and KJ812200 (ITS) and OL435812 (*trnL-trnF*) for the San Marcos River plants.

Cuscuta obtusiflora, commonly known as Peruvian dodder, is native to much of the Americas (POWO 2021). The variety known as *C. obtusiflora* var. *glandulosa* (≡ *C. glandulosa* (Engelm.) Small) has a more northern distribution than var. *obtusiflora*, and the two varieties are allopatric in North and South America, respectively. In the U.S.A., *C. obtusiflora* var. *glandulosa* is concentrated mostly in the Gulf Coast region with limited distribution in the Northeast and California (Kartesz 2015).

Phylogenetically, var. *glandulosa* and var. *obtusiflora* are distinct lineages on both the ITS and *trnL-trnF* trees (Costea et al. 2015b), and thus data for either ITS or *trnL-trnF* would be sufficient to identify an unknown specimen. *Cuscuta* species have been sampled thoroughly in phylogenetic analyses, and in fact every North American species is represented by both ITS and *trnL-trnF* data (Stefanović et al. 2007; Costea et al. 2015a, 2015b), so the Texas plants are definitively identified as *C. obtusiflora*. Peruvian dodder belongs to *Cuscuta* subg. *Grammica* (Lour.) Peter sect. *Cleistogrammica* Engelm. and is a close relative of other North American species, including *C. campestris* Yunck. and *C. polygonorum* Engelm. (Costea et al. 2015a).

Morphologically, *C. obtusiflora* plants can be identified by the presence of densely fringed infrastaminal scales and corolla lobes that are obtuse and straight (i.e., not inflexed at the apices) (Small 1903; Costea et al. 2006; Spaulding 2013). However, translucent laticifers that sometimes are purported to distinguish *C. obtusiflora* (e.g., Engelmann 1859; Small 1903) actually are widespread throughout the "*C. pentagona* complex" and not unique to that taxon (Costea et al. 2006). Flowers were not observed on any submersed *Cuscuta* plants in our study, however in the same localities in 2013, when water levels had fallen, we observed *C. obtusiflora* growing and flowering on emergent *H. polysperma* (Fig. 2). We also noted that axillary shoots of *C. obtusiflora* growing on submersed *H. polysperma* occasionally would emerge above the water surface during low water flows.

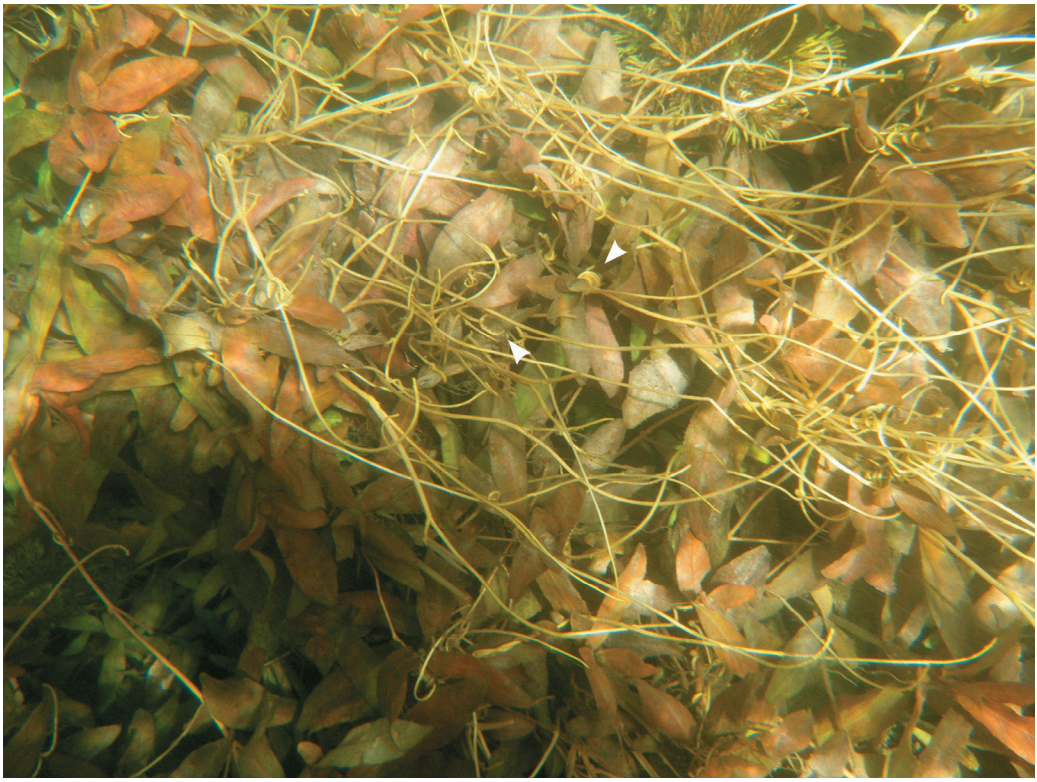

Fig. 1. *Cuscuta* plants (slender strands) interacting with *Hygrophila polysperma* (broad leaves and associated stems), while both plants are completely underwater. Two instances of haustoria are highlighted with white arrows, visible as outgrowths from a tightly wound portion of *Cuscuta* stem.

Nineteen different *Cuscuta* species have been implicated as associates of obligate wetland plants in North America (Les 2018, 2020), and direct parasitism of wetland obligates has been recorded for six host species. Peruvian dodder is a frequent parasite on hydrophytes, preferring members of the genus *Polygonum* L. (= *Persicaria* (L.) Mill. pro parte) as hosts (Engelmann 1859; Yuncker 1932; Godfrey & Wooten 1981) as well as *Justicia americana* (Correll & Correll 1972). The *C. obtusiflora* plants growing in the San Marcos and Comal rivers were found to occur almost entirely on *H. polysperma*, a federally listed noxious aquatic weed (USDA APHIS 2012) and a common macrophyte in both systems (Williams 2019), although we observed them rarely on adjacent native *Ludwigia repens* J.R.Forst. as well. It will be important to investigate the life history of the submersed *Cuscuta* in the observed populations, to determine its mechanisms of establishment, spread, and persistence in an aquatic environment.

Cuscuta species grow occasionally in aquatic environments, including riparian and wetland habitats (Spaulding 2013; Costea et al. 2016). In section *Cleistogrammica* and related lineages, hydrochory is facilitated by capsules that are indehiscent and remain buoyant for several days (Ho & Costea 2018). Moreover, plants in this group successfully germinate after endozoochory by waterfowl (Costea et al. 2016; Olszewski et al. 2020). Whereas *Cuscuta* species overwhelmingly parasitize terrestrial or emergent wetland plants, there have been few observations of underwater growth in the genus. Glück (1911) recorded instances of *C. alba* J. Presl & C. Presl (≡ *Cuscuta epithymum* (L.) L. var. *alba* (J. Presl & C. Presl) Trab.) growing submersed under experimental conditions, for which he named C. *alba* forma *submersa* Glück (Arber 1920). Later, Gaertner (1950) noted

Fig. 2. *Cuscuta* plants interacting with emergent *Hygrophila polysperma* during a period of low water levels. Under these conditions the *Cuscuta* plants were able to flower.

the potential for *C. suaveolens* (a species with indehiscent fruits in sect. *Racemosae*; Ho & Costea 2018) to thrive during temporary submergence while parasitizing the obligate wetland plants *Alternanthera philoxeroides* (Mart.) Griseb. and *Nasturtium officinale* W.T. Aiton (Ashton & Santana 1976). After identifying the submersed dodder in Texas, we were alerted to the existence of another herbarium specimen of *C. obtusiflora* collected in California in 1995 (*Barbe 4466*) and parasitizing *Myriophyllum sibiricum* Kom. (M. Costea, pers. comm.).

Our record confirms for the first time with molecular evidence that *C. obtusiflora* plants can grow completely submersed, at least seasonally, while parasitizing aquatic macrophytes. Because Peruvian dodder plants are not known to be predominantly aquatic, we infer that they are able to become established on emergent *H. polysperma* during periods with low water levels and persist underwater as river levels rise.

Voucher specimens: **U.S.A. CALIFORNIA. Sacramento Co.:** Elk Grove, Laguna Lake, N of Elk Grove Blvd. and W of Foulks Ranch Dr., 38.414491 N, 121.432946 W, 2 Jun 1995, *G.D. Barbe 4466*, CDA0009367, CHSC79721, HSC94033, OBI125996, RSA671135, UC1778597. **TEXAS. Comal Co.:** New Braunfels, Comal River, collected in the Old Channel growing submersed on *Hygrophila polysperma* in 2 to 3 ft of water, gently flowing, 29°42'44.83"N, 98°07'37.72"W, 13 Jan 2013, *Williams 249* (SWT). **Hays Co.:** San Marcos, San Marcos River, between "Icehouse" and dam, 1 Jul 1984, *Ertter 5486* (TEX00394269); San Marcos, San Marcos River, collected above I-35 bridge in marshy backwater growing on emergent *Hygrophila polysperma*, 4 Sep 2009, *Williams 64* (SWT); San Marcos, San Marcos River, collected along San Marcos at Hell's Half Acre Island growing on *Hygrophila polysperma*, fully submerged, 29°52'53.10"N, 97°56'02.96"W, 18 May 2010, *Williams 107* (BRIT); 25 Apr 2013, *Williams 256* (BRIT).

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