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INVASION NOTE

Invasion of *Nipponaclerda biwakoensis* (Hemiptera: Aclerdidae) and *Phragmites australis* die-back in southern Louisiana, USA

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Abstract Common reed, *Phragmites australis* (Cav.) Trin. Ex Steud., is the dominant emergent vegetation in the lower Mississippi River Delta (MRD), Louisiana, USA and is comprised primarily of introduced lineages of different phylogeographic origins. Dense stands of *P. australis* are important for protecting marsh soils from wave action and storm surges. In the Fall of 2016, while investigating symptoms of die-back of *Phragmites* stands in the lower marsh, a non-native scale was found infesting affected stands in high densities. Identified as *Nipponaclerda biwakoensis* (Kuwana) (Hemiptera: Aclerdidae), the scale was well established across the lower MRD. This report represents the first recorded population of *Nipponaclerda biwakoensis* in North America. Intriguingly, there are noticeable differences in die-back symptoms and in scale densities among different lineages of *Phragmites* in the MRD, with stands of the well-known European invasive lineage appearing healthier and having lower scale densities than other *Phragmites* lineages. Given its apparent relationship with the die-back syndrome, the scale may have serious implications for the health and stability of *Phragmites* marsh communities across

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coastal Louisiana. Efforts are currently underway to investigate the role of the scale and other abiotic stressors in the die-backs and potential management solutions.

Keywords *Phragmites* lineages · Scale insect · Mississippi River Delta

Common reed, *Phragmites australis* (Cav.) Trin. Ex Steud. (hereafter referred to as *Phragmites*), is a cosmopolitan perennial grass found in fresh to salt water marshes. *Phragmites* can provide several ecosystem services including carbon sequestration, water quality maintenance, and food and habitat resources for wildlife (reviewed in Kiviat 2013). While native lineages are found across North America, invasive lineages from Europe have expanded into coastal wetlands over the past 150 years, replacing diverse plant communities with dense monocultures (Chambers et al. 1999; Rice et al. 2000; Saltonstall 2002). In the outer marshes of the Mississippi River Delta (MRD) in Louisiana, USA, *Phragmites* is the dominant emergent vegetation and is considered critical for trapping sediments, stabilizing soils, and protecting the interior marsh from wave action and storm events (Coops and Van der Velde 1996; Rooth and Stevenson 2000; Horppila et al. 2013).

The MRD is a hotspot for *Phragmites* genetic diversity, with six lineages present (Howard et al. 2008; Hauber et al. 2011; Lambertini et al. 2012). The Land-type (haplotype I or subspecies *P. australis berlandieri*) is found throughout Central and South America (Saltonstall 2002; Colin and Eguiarte 2016). In North America, Land-type (also known as the Gulf Coast lineage) is widespread along the Gulf Coast and has recently spread southwest to California (Saltonstall 2002; Howard et al. 2008; Meyerson et al. 2010; Hauber et al. 2011). The Delta-type (haplotype M1) is of North African/Mediterranean origin and is the dominant haplotype in the MRD (Hauber et al. 2011; Lambertini et al. 2012). The EU-type (haplotype *M* or subspecies *P. australis australis*) is of European origin, invasive throughout North America, and present in scattered stands within the MRD (Lambertini et al. 2012). A complex of three haplotypes (M, AD, and AI or Greeny 1, Greeny 2, and Greeny 3, respectively), collectively referred to as Greeny, appears to be the rarest in the MRD (Lambertini et al. 2012). Finally, we note that none of the endemic North American native haplotypes identified by Saltonstall (2002) have been found in the MRD or Gulf Coast of the USA.

In the Fall of 2016, die-back of *Phragmites* stands in the lower MRD was reported by concerned land owners. While the symptoms of die-backs became severe enough to warrant the concerns of land managers, multi-year analysis of NDVI from satellite imagery of the MRD suggests the die-backs may have been occurring for several years (Ramsey and Rangoonwala 2017). Die-back of *Phragmites* is not a new phenomenon, with reports from Europe (van der Putten 1997), China (Li et al. 2013), and North America (McDonald 1955). In Europe, die-back was attributed primarily to eutrophication and accumulation of phytotoxins like sulphide in the sediments (e.g., Armstrong et al. 1996b; van der Putten 1997). Previous die-backs in North America were attributed to higher than usual water tables in the winter resulting in the death of dormant shoots (McDonald 1955). Natural enemies may also play a role as insect-bore holes, and infestation by aphids and fungal pathogens have been shown to reduce airflow in *Phragmites* stems and are linked to premature senescence and dead stems (Armstrong et al. 1996a). Die-backs are of particular concern in the lower delta as loss of *Phragmites* could result in reduced sedimentation and sediment infilling of navigation channels (Temmerman et al. 2012).

While investigating *Phragmites* die-backs in the Pass-a-Loutre Wildlife Management Area (29.09, − 89.13), Louisiana, affected patches were found to have high population densities of a scale insect. This scale was identified as *Nipponaclerda biwakoensis* (Kuwana) (Hemiptera: Aclerdidae) (hereafter referred to as *Nipponaclerda*), an introduced species found in China and Japan, and represents the first record of an established population of the species in the United States. Identification was confirmed through morphological characteristics (McConnell 1953) and through sequencing of the mitochondrial COI barcoding region using the PcoF1 and LepR1 primer sets (Park et al. 2010). Mitochondrial DNA from eight individual scales collected from Louisiana (GenBank accession nos. MH169002–MH169009) are 99.8% identical to sequence data for *Nipponaclerda biwakoensis* in

GenBank (accession no. KP189751), with a single transition from T to C at nucleotide position 153.

While there are approximately 64 scales or scale-like insects associated with *Phragmites* (García Morales et al. 2016), only *Chaetococcus phragmitis* (Marchal) (Hemiptera: Pseudococcidae) and *Eriopeltis festucae* (Boyer de Fonscolombe) (Hemiptera: Coccidae) were reported on *Phragmites* in North America prior to *Nipponaclerda* (see Tewksbury et al. 2002). Both species are found throughout Europe and are likely introduced to North America (Kosztarab 1996; García Morales et al. 2016). While monitoring *Nipponaclerda* populations in Louisiana, we have discovered an additional scale species, *Aclerda holci* Teague (Hemiptera: Aclerdidae) on *Phragmites*. *Aclerda holci* is native to Louisiana and found on Johnsongrass, *Sorghum halepense* (L.) Pers. (Ferris 1955). It is also recorded as a non-economically important insect on sugar cane, *Saccharum officinarum* L. (Meagher and Gallo 2008). The USNM National Entomology Collection also houses specimens of *A. holci* collected on *Arundo donax* L., *Cynodon dactylon* (L.) Pers., and *Sporobolus wrightii* Munro ex Scribn.

Visual differentiation of mature female scales between the two species is generally straightforward. Both species, as aclerdids, lack wax except around the margins (McConnell 1953). Adult females of *A. holci* can be distinguished by their pointed and partially sclerotized abdomen from *Nipponaclerda* which are more uniformly sclerotized with a rounded abdomen (Fig. 1). Also, *A. holci* only appears to occur at densities of a few individuals per stem, while *Nipponaclerda* infestations can reach over two thousand scales per stem (R. Diaz pers. obs.). Superficially, *Nipponaclerda* might be confused with *C. phragmitis*, which also has a rounded abdomen. *C. phragmitis* can be distinguished by the distinct segmentation of posterior abdominal segments, which *Nipponaclerda* lacks. In the United States, *C. phragmitis* is only known to occur in the mid-Atlantic region (Kosztarab 1996).

Nipponaclerda settle under the leaf sheaths of *Phragmites* stems and are found in reed wetlands across Japan, China, and Korea (Kuwana 1907). In addition to *Phragmites,* reported hosts of *Nipponaclerda* include members of the genera *Agropyron* and *Juncus* (Wang 1994). In its native range, *Nipponaclerda* is reported as having between 3 and 6

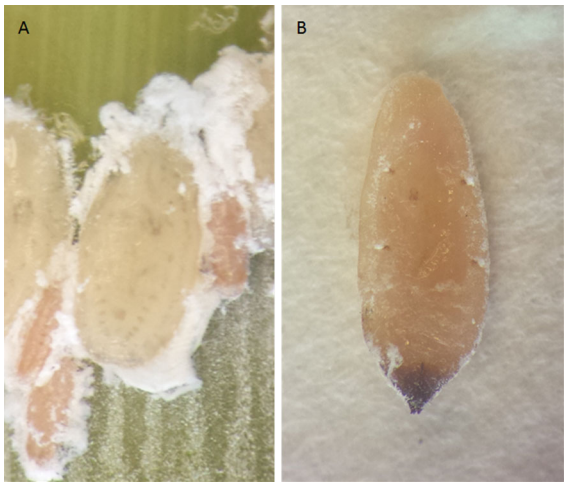

Fig. 1 Side by side comparison of **a** invasive *Nipponaclerda biwakoensis* and **b** native *Aclerda holci*. Both specimens were collected from *Phragmites australis* in Louisiana. Note the sclerotized posterior of *A. holci*; this is the primary characteristic for distinguishing adult female scales in the field

generations per year (Xia et al. 1993; Kaneko 2004). The scales overwinter as nymphs and adults, and the first generation nymphs are observed infesting new shoots in May in China, but were observed as early as March in Louisiana. In natural systems across its native range, *Nipponaclerda* can represent up to 83 percent of arthropods in *Phragmites* stands and is an important food source for endemic bird species like the Reed Parrotbill, *Paradoxornis heudei* David (Gan et al. 2010). *Nipponaclerda* does not appear to have major impacts on *Phragmites* growth and survival in its native range and has received little attention outside of its use as a model system for parasitoid studies (Kaneko 2004, 2005a, b). During the summer of 2017, *Nipponaclerda* females collected in Louisiana were parasitized by *Neastymachus japonicus* Tachikawa, *Boucekiella depressa* Hoffer, and *Astymachus* sp. Howard (Hymenoptera: Encyrtidae). The native range of *N. japonicus* is Japan, and *B. depressa* has been reported in Europe, Asia, and North America (Noyes 2017).

With potential parasitism rates as high as 45% and reductions in overwintering female scales owing to bird predation up to 85% (Kaneko 2004, 2005b; Xiong et al. 2010), natural enemy pressure appears sufficient to suppress *Nipponaclerda* in their native range. However, *Nipponaclerda* is considered a pest in China where *Phragmites* wetlands are managed for

production of reed biomass for the paper industry (Brix et al. 2014). In addition to reliance on natural enemies, management of *Nipponaclerda* includes cutting stems, which reduces scale density without affecting total parasitism rates (Ma et al. 2014); inundation of stands in early winter which reduces survival of overwintering scales (Qin et al. 2003); and burning away reed residues following harvest, a practice known as Shaotang, which reduces scale infestations but may result in nitrogen deficiencies (Shen et al. 1995; Brix et al. 2014).

Current efforts are underway to assess the distribution and impact of *Nipponaclerda* in Louisiana's coastal marshes. As of September 2017, *Nipponaclerda* has been recorded from 14 parishes in southern Louisiana (Fig. 2). While primarily established in the southeastern parishes along the Mississippi river, the scale has been detected further west along the Gulf coast. This distribution appears to coincide with regions experiencing *Phragmites* die-back; however, the role of *Nipponaclerda* and its interaction with other stressors is currently under investigation.

In addition to mapping the distribution of the scale and studying its role in the die-backs, efforts are underway to map the extent of the die-backs using satellite imagery and identify stands of resistant *Phragmites*. Although all four lineages (Delta, Gulf, EU and Greeny) have been observed to be attacked by *Nipponaclerda*, in comparisons between contiguous Delta and EU stands, scale densities were 8 times higher on the former than latter lineage based on a mid –summer survey in 2017 (J.T. Cronin pers. obs.). This result is consistent with the published literature that European invasive *Phragmites* is more resistant to herbivores than other lineages of *Phragmites* (Lambert and Casagrande 2007; Lambert et al. 2007; Allen et al. 2015; Cronin et al. 2015; Bhattarai et al. 2017). Whether *N. biwakoensis* or *A. holci* can be considered potential biological control agents of invasive populations of EU-type *Phragmites* or pests of important

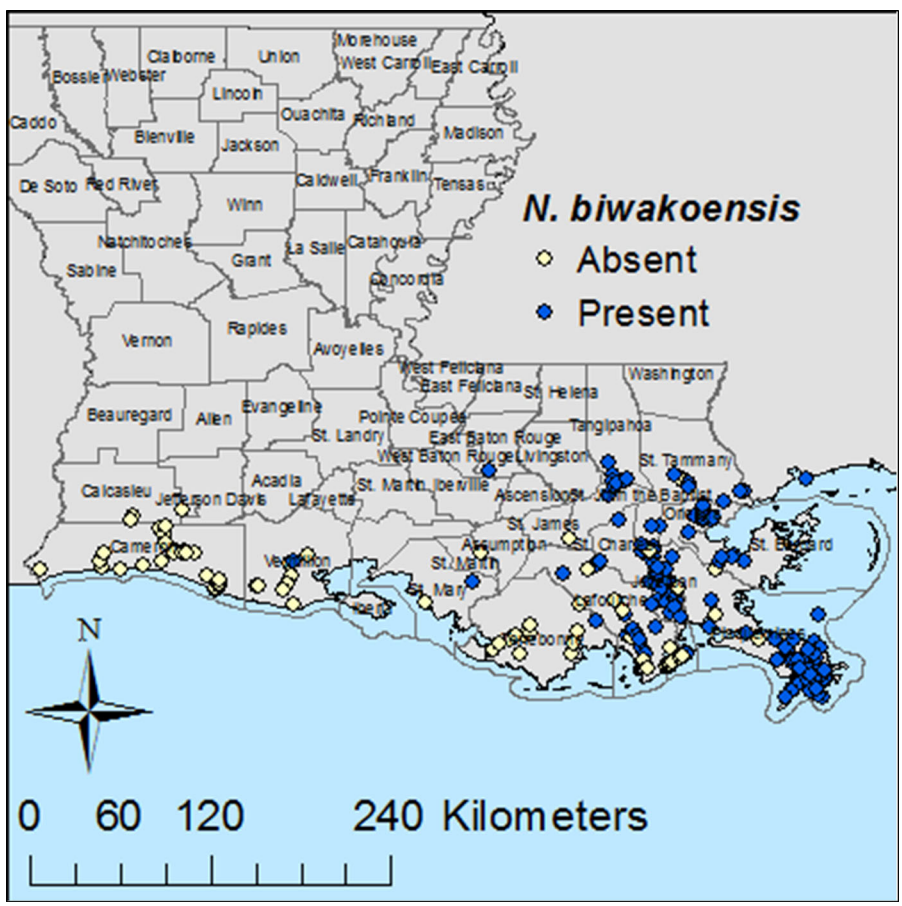

Fig. 2 Distribution map of *Nipponaclerda biwakoensis* as of October 2017. All sample points represent patches where *Phragmites* is present. Sites were sampled at least once from Fall, 2016 through Fall, 2017

grasses remains unknown, ongoing host specificity studies will solve this uncertainty.

The different lineages present in the MRD may be of some benefit to mitigation of the potential implications of *Phragmites* die-back. In addition to the European lineage being more resistant to a diverse herbivore assemblage (see above), it generally has broader tolerances to a wide range of environmental conditions including salinity (Vasquez et al. 2005; Achenbach and Brix 2014; Eller et al. 2017). If left alone, we might expect the European lineage to expand its range and relative abundance within the MRD as the Delta lineage continues to decline. A more heretical viewpoint, given the aggressively invasive nature of the European lineage, is that it be used specifically as source material for restoration efforts. In light of the staggering loss of coastal habitat in Louisiana, 75 km^2 per year (Williams 1995), the rewards may outweigh the risks in using this approach.

The extent of the role of *Nipponaclerda* in the die-back and how the scale will affect the long term health and structure of the marsh is unknown. Initial observations suggest that the die-back syndrome is widely distributed across the MRD and concurrent with the scale; however, the geographic extent of the scale goes beyond the MRD including regions where die-back symptoms are less apparent. Currently, efforts are underway to investigate how *Nipponaclerda* is affecting the health of *Phragmites* stands in the lower MRD, resistance of *Phragmites* lineages to the scale and salt intrusion, the risk of the scale to native and agriculturally important grass species in Louisiana, and chemical management options for the scale. Voucher specimens of *Nipponaclerda* and *A. holci* were deposited in the US National Museum of Natural History, Louisiana State Arthropod Museum (LSAM), and parasitoids were deposited in the British Natural History Museum and LSAM.

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