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Suitability of Introduced *Nymphoides* spp. (*Nymphoides cristata, N. peltata)* as Targets for Biological Control in the United States

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PURPOSE: This technical note summarizes the results of a scoping study to determine whether the introduced *Nymphoides cristata* and *N. peltata* are suitable targets for classical biological control in the United States. A thorough literature review of biology, management, and impacts of both *Nymphoides* spp. was conducted, and the standard Peschkin-McClay scoring system was applied to assess their suitability as classical biological control targets. Scores from independent reviewers were averaged; 146 for *N. cristata* and 148.5 for *N. peltata*. Based on these scores, it was concluded that these *Nymphoides* spp. are good candidates for biological control. Presented here is the justification for the Peschken-McClay scores and the results of climate-matching studies to determine overseas locations where initial exploration for agents should take place.

INTRODUCTION: Aquatic invasive plants require substantial management costs each year with the goal of preventing or mitigating their associated environmental and economic impacts (Wainger et al. 2018a, Wainger et al. 2018b). Management approaches may include a combination of herbicide application, physical removal, resource competition through restoration, and biological control using host-specific insect herbivore or pathogen agents. Of these, biological control offers a potentially self-sustaining solution that can reap long-term economic and ecological benefits (Wainger et al. 2018a, Wainger et al. 2018b).

Classical biological control of weeds involves the use of host-specific organisms to suppress target populations below some damage threshold (Coombs et al. 2004). Biological control often includes the use of co-adapted insect herbivores (biological control agents), sourced from populations in the native range of the pest species. When introduced into the exotic range, these biological control agents are released without their own parasites or predators, which allows for rapid population buildup of the agent and, potentially, suppression of the target weed. Prior to initiation of a biological control program, however, it is important to determine the likelihood of success (Peschken and McClay 1992, Pemberton 1996, Pemberton 2002), which may inform whether the focal pest is a suitable target. This is especially relevant given the high cost and potentially long-term nature of biological control programs (Andres 1977, Paynter et al. 2015).

Assessments to determine biological control target suitability should be based on biological (e.g., climatic tolerances, reproductive strategy of the weed, known natural enemies) and economic (costs associated with damages or management) criteria related to the severity of the pest and likelihood for suppression using biological control. A scoring system to meet this need was developed by McClay

(1989) and further refined by Peschken and McClay (2009) and is a semi-quantitative method for objectively assessing potential weed targets for biological control (Cuda and Sutton 2000).

Nymphoides cristata (Figure 1) and *N. peltata* (Figure 2) are introduced floating-leaved plants native to Asia (*N. cristata*) and Eurasia (*N. peltata*) and have been present in the U.S. since 1996 and 1882, respectively (Les and Mehrhoff 1999, Burks 2002b). Impacts from dense infestations come from competition with beneficial species for nutrients, shading of submersed species, reduction of dissolved oxygen, reduced habitat quality for aquatic animals, reduced boating and recreational access (Burks 2002b, DCR 2011), and reduced flow. To date there has not been an attempt to develop biological control agents for either *Nymphoides* species in the U.S. The objective of the current technical note is to summarize the results of an assessment to determine whether introduced *Nymphoides* spp. in the U.S. are appropriate targets for classical biological control and to provide recommendations for a new biological control program. Here, the results of Peshkin-McClay (P-M) scores for each species are reported then compared with previous assessments, providing supporting information that led to conclusions for each scoring category. The authors conclude by recommending the first steps in initiating a biological control program and report the results of a climate-matching study to prioritize locations to search for biological control agents in the native range of each species.

Figure 1. *Nymphoides cristata* colony in Lake Marion, SC.

Figure 2. A native population of *Nymphoides peltata* in Shijidiaosu Park, China (left), and an introduced population in Lake Dallas, TX (right).

RESULTS AND DISCUSSION

Peschkin-McClay score. Based on assessment by two reviewers, we calculated average P-M scores of **146** for *N. cristata* and **148.5** for *N. peltata* (Table 1) out of an available score of **179**. P-M scores for each species are similar to those calculated for weeds previously considered to be good candidates for biological control, such as *Matricaria perforata* (Peschken et al. 1988, McClay and De Clerck-Floate 1999). For introduced *Nymphoides*, scores would have been higher if they were previous targets of biological control or more was known about natural enemies in the native ranges. Economic losses were scored in the mid-range, which mainly represents a lack of knowledge surrounding economic impacts from *Nymphoides* infestations. However, losses from aquatic invasive plants can be substantial, especially when they limit navigation or water delivery and require constant management to prevent impacts (Wainger et al. 2018a, 2018b). Additionally, herbicide use can be tailored to minimize — but rarely eliminate — off-target effects in many cases, so scores were moderate in that category (Table 1, A5- Available means of control: Environmental damage). General information used to assign scores is given in the next section and presented by species.

Table 1. Summary of scores for *N. cristata* and *N. peltata*, using the Peschkin-McClay scoring system to evaluate suitability of targeting these species for biological control in the U.S. Maximum score (without known biological control agents) is 179.

	Average score	
Category	***N. cristata***	***N. peltata***
A. ECONOMIC ASPECTS		
1. Economic losses	20	20
2. Infested area	5	7.5
3. Expected spread	10	10
4. Toxicity	0	0
5. Available means of control		
Environmental damage	10	10
Economic justification	10	10
6. Beneficial aspects	0	0
7. Biological aspects		
Infraspecific variation	10	10
8. Native range	30	30
9. Relative abundance	10	10
10. Success elsewhere	0	0
11. Number of known agents	0	0
12. Habitat stability	30	30
13. Economic species in genus	3	3
14. Economic species in tribe	4	4
15. Ornamental species in genus	0	0
16. Ornamental species in tribe	1	1
17. Native species in genus	1	1
18. Native species in tribe	2	2
TOTAL	**146**	**148.5**

Nymphoides cristata

Economic Losses. Current costs of *N. cristata* management or damage within North America are not available as the species is a relatively new introduction. Mechanical removal of *N. peltata* in Sweden was estimated to cost $9,000 (USD) per hectare annually (Gren et al. 2007). Thus, based on this estimate, it would cost approximately $21.6 million to mechanically remove the entire 2,400 ha infestation present in Santee Cooper reservoir in South Carolina. Mechanical harvesting is likely to be a cost-prohibitive management technique in large infestations and reinfestation is likely to occur.

Nymphoides cristata infestations have been documented to impede waterbody navigation within the southeastern U.S. (Gettys et al. 2017), which can have far-reaching economic impacts through subsequent loss of recreation and tourism. The American Sportfishing Association and the National Marine Manufacturers Association estimate recreational angling and boating contribute $115 billion and $121.5 billion annually to the U.S. economy, respectively (NMMA 2013, Southwick 2015). Any negative impacts to either of these activities could be economically significant.

Infested Area. Although *N. cristata* is mostly a regional problem (confined to the southeastern U.S.), infestations can be substantial. For example, *N. cristata* infestations in Santee Cooper Reservoir, South Carolina, cover approximately 2,400 ha (Willey et al. 2014). Currently, *N. cristata* is widely distributed in Florida, and scattered populations occur in Texas, South Carolina, and North Carolina (Nault and Mikulyuk 2013).

Expected Spread. The U.S. Department of Agriculture (USDA) conducted Weed Risk Assessments (WRA) for *N. cristata* in 2012 and concluded that the Southeastern U.S. was suitable for establishment. There is high probability of spread from current infestations. The USDA WRA reports a 66% probability that *N. cristata* will become a major invader and a 22% chance of it becoming a minor invader (USDA 2012a).

To complement the conclusions of the USDA WRA, the team conducted a climate-matching study (details on process can be found in the below section, "Biological control implementation"), using known occurrences of *N. cristata* in its Asian range to predict climate suitability of locations in the U.S. *Nymphoides cristata* is likely to establish and persist primarily in the Southeastern U.S. (Figure 3), which is in agreement with the USDA assessment (USDA 2012a). There are not many known populations outside Florida, but there is a likelihood of spread and establishment from known locations.

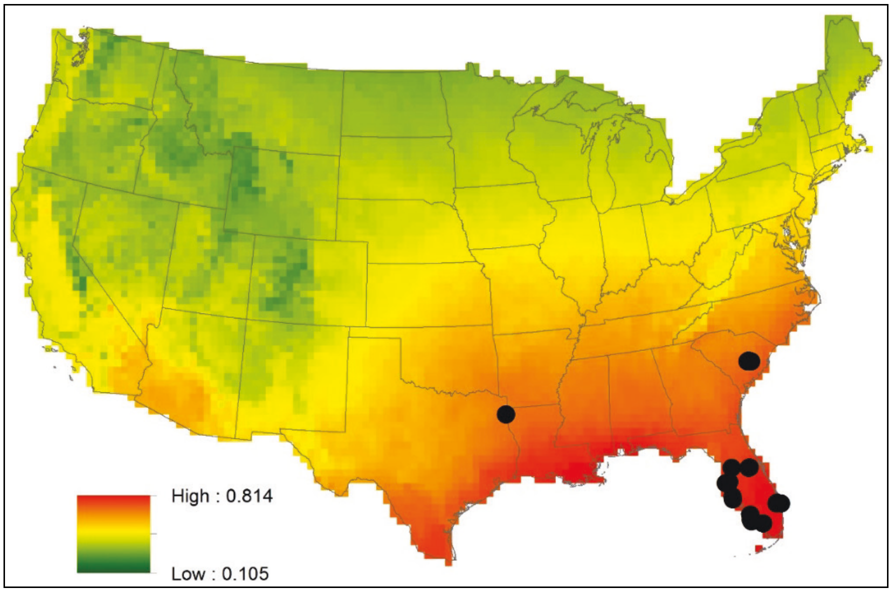

Figure 3. Results of climate matching for *Nymphoides cristata*. Known occurrences of *N. cristata* from Asia were used to predict locations in the U.S. where *N. cristata* could survive, due to similarity in climate conditions. Black dots are occurrences from EddMapS.org and do not include more recent records from Louisiana and North Carolina.

Toxicity. This plant is apparently non-toxic to wildlife (Burrows and Tyrl 2012).

Available means of control

Mechanical. Mechanical harvesting is expensive (Burks 2002a, Gren et al. 2007) and often ineffective for species that reproduce vegetatively, as fragments generated during the process may contribute to new infestations (Burks 2002a). In a controlled study in which shoots were clipped and removed, clipped plants recovered quickly and produced the same biomass as unclipped plants (Middleton 1990).

Biological control. There are currently no known insect biocontrol agents, and triploid grass carp do not consume *N. cristata* (Van Dyke et al. 1984, Burks 2002a).

Herbicides. Herbicide trials are currently ongoing; however, in general, *N. cristata* is considered difficult to control because it is broadly tolerant to most registered herbicides (Willey 2012). However, recent advances in herbicide development has proven promising; Beets and Netherland (2018) demonstrated strong control of *N. cristata* with florpyrauxifen-benzyl and limited impacts to native non-target species. Traditionally, the primary obstacle to effective herbicide treatment of *N. cristata* is the frequency with which surface leaves dip below the water's surface when disturbed by wind or wave action (Willey et al. 2014), thus removing and diluting herbicide applied to the leaves. However, Mudge and Netherland (in review) found that subsurface application of florpyrauxifen-benzyl was effective in controlling *N. cristata* in a mesocosm experiment. In addition to recent advances with florpyrauxifen-benzyl, the most promising herbicide options include submersed applications of diquat and endothall; foliar applications of imazapyr, imazamox, and endothall; and foliar combinations of flumioxazin and glyphosate (Glomski et al. 2014, Willey et al. 2014, Mudge and Netherland in review).

Environmental damage caused by control methods. Mechanical harvesting and herbicide use are both generally considered environmentally safe within North America, but both may impact non-target vegetation, including native, beneficial plants.

Economic justification of control methods. Mechanical harvesting is most likely cost-prohibitive. A report from Sweden estimated costs at $9,000 US per hectare to remove *N. peltata*, a closely related species (Gren et al. 2007). In addition, mechanical harvesting may contribute to further spread by propagule generation during the removal process (Madsen 2000). Current cost estimates for long-term management of the plant are not available and vary based on size of infestation and success rates of available herbicide formulations. In general, herbicides are considered affordable within North America.

Beneficial aspects. Within North America there are five native *Nymphoides* that occupy a similar ecological niche as *N. cristata*. These species are found in aquatic habitats spanning a great distance: from the Caribbean islands, Guatemala, and Mexico to the eastern regions of the United States. The introduction and continued presence of *N. cristata* in North America does not provide any additional environmental benefits that are not already provided by native *Nymphoides* species or other native macrophytes. In contrast, the invasion of *N. cristata* has been shown to form monotypic stands that outcompete native vegetation (Willey and Langeland 2011), reduce water flow and aeration (Burks 2002a), and impede recreational activities on waterbodies (Gettys et al. 2017). Because there are no known positive benefits of *N. cristata,* there are no conflicts of interest regarding initiation of a biological control program.

Biological aspects

Infraspecific variation. *Nymphoides cristata* is reportedly spread by vegetative reproduction in the U.S. and no viable seeds have yet been observed (Burks 2002b). However, there is some concern about hybridization between *N. cristata* and native *Nymphoides* (L. Gettys, personal communication[1]) in Florida and South Carolina where they co-occur. It will be critical in the early stages of biological control development to determine whether hybridization occurs between invasive and native species. If hybridization occurs, it may limit the options for importing and releasing biological control agents in the U.S. because the chances of biological control agents adapting to feed on non-target native species through a hybrid-intermediary increases (Floate and Whitham 1993). Despite the lack of viable seeds in the U.S., *N. cristata* produces abundant ramets, which can readily break free of the parent plant and establish new colonies elsewhere (Burks 2002b). Through vegetative means, *N. cristata* can form a new plant from fragments, rhizomes, or daughter plants (Burks 2002a, Willey and Langeland 2011). Ramets, a cluster of rhizomes, are produced at the junction of every leaf-petiole in *N. cristata* (Gettys et al. 2017).

[1] L. Gettys.2017. Personal communication with Nathan Harms via telephone. October 15.

Native range. *Nymphoides cristata* is native to Asia, and found in southeastern China, India, Taiwan, Indonesia, Myanmar, Thailand, and Vietnam (GBIF.org 2017). Its native range may include a number of other Southeast Asian countries as well and is probably underreported.

Relative abundance. There is little information available regarding invasiveness of *N. cristata* in its native range, though it has been regarded as a pest of rice fields (Burks 2002a). The invasive potential of *N. cristata* in introduced areas is well-documented and is due to its varied means of reproduction coupled with a strong competitive ability. In studies of ramet production in moderate sediment fertility, a single *N. cristata* plant produced 350 ramets in six months, a figure that was used to estimate a production potential of 49,000 ramets in a single year from first generation progeny (Gettys et al. 2017). This plant has been observed to densely cover a 3.6-hectare lake in approximately one month (Burks 2002a). *Nymphoides cristata* is tolerant of short freezes and shallow (0.6m) and moderately deep (3m) water (Willey et al. 2014). *Nymphoides cristata* successfully regrows from the root crown following desiccation (Mason 1996).

In addition to highly successful vegetative propagation, *N. cristata* is thought to be an effective competitor. Competition trials were conducted between *N. cristata* and *Hydrilla verticillata*, a significant North American invasive species. It was demonstrated that *N. cristata* was the superior competitor in that the effect of competition on *H. verticillata* was much stronger than on *N. cristata* (Willey 2012). Only in treatments planted with five-fold more *H. verticillata* was biomass of *N. cristata* reduced substantially from that grown alone (Willey 2012). *Nymphoides cristata* also consistently elongated and reached the water's surface at more rapid rates than *H. verticillata* (Willey 2012).

Success of biological control elsewhere. Biological control of *Nymphoides* spp. has not been attempted elsewhere.

Number of known promising control agents. Thus far there are no known potential agents. There are a number of species reported from native *Nymphoides* in the U.S. (Harms and Grodowitz 2009) and *N. peltata* in its native range (see below). Percent herbivory has been previously reported for *N. cristata* from monsoonal wetlands in India, but in that case only damage was recorded and not the responsible organisms (Mason 1996). However, herbivory of up to 40% leaf damage was apparently responsible for significant declines (68% reduction in standing biomass) in *N. cristata* populations, which is promising if effective biological control agents are eventually discovered for use in the U.S. In contrast, Middleton (1990) found no difference in total biomass between clipped and unclipped *N. cristata* in an attempt to simulate goose herbivory.

Habitat stability. *Nymphoides* occur mainly in permanently wet environments. Although plants can survive in damp conditions, *N. cristata* benefits from locations with annual drawdowns, apparently due to enhanced survival of seedlings under drawdown conditions (Mason 1996). However, mature plants do not survive drying conditions, and since *N. cristata* is thought to reproduce only vegetatively in the U.S., it likely requires permanently moist conditions to persist.

Number of economic species in the genus Nymphoides. There are no *Nymphoides* species of economic importance present within North America.

Number of economic species in the family Menyanthaceae. There are no species of economic importance within the Menyanthaceae family within North America.

Number of ornamental species in the genus Nymphoides. There are currently at least six ornamental *Nymphoides* species commercially available within North America. One species, *N. aquatica*, is native to North America, while the other five species are non-native (*N. peltata*, *N. geminata*, *N. cristata*, *N. indica*, and *N. crenata*). *Nymphoides peltata* and *N. cristata*, the focal species in this assessment, are also problematic invasive exotic plants.

Number of ornamental species in the family Menyanthaceae. In addition to the six ornamental *Nymphoides* species listed above, *Menyanthes trifoliata* is available commercially in North America. The remaining three genera within the family Menyanthaceae (*Liparophyllum*, *Nephrophyllidium*, and *Villarsia*) were investigated, but commercial availability was not confirmed.

Number of native North American species in the genus Nymphoides. There are five *Nymphoides* species native to North America. Three species are native to various regions of the United States. *Nymphoides aquatica* is distributed in the southeastern United States, *N. cordata* in the eastern, and *N. humboldtiana* is unique to Texas and potentially locations in the Caribbean where it may be misidentified and recorded as *N. indica* (Tippery et al. 2011, Tippery et al. 2015). Additionally, *N. fallax* is native to high-altitude regions of Mexico and Guatemala, and *N. grayana* is native to the Caribbean (Ornduff 1969, 1970, Burks 2002a, Tippery et al. 2015).

Number of North American species in the family Menyanthaceae. In addition to the five North American native *Nymphoides* species listed above, two other species in the Menyanthaceae family are present in North America. *Menyanthes trifoliata* is present in North America between 40° north and the Arctic Circle, and *Nephrophyllidium crista-galli* is located along the Pacific Coast (Kadereit and Jeffrey 2007).

Nymphoides peltata

Economic Losses. Similar to *N. cristata*, current management or damage costs for *N. peltata* within North America are not available. Thus, potential economic impacts are similar to *N. cristata*. However, because *N. peltata* has a much larger geographic distribution in the U.S., it can be anticipated that the actual impacts will exceed those from *N. cristata*. *Nymphoides peltata* has been documented to completely halt recreational lake activities leading to property value decline (DCR 2011) and loss of recreational or tourism income. Although it is difficult to attribute financial costs to loss of ecosystem goods and services, the overwhelming evidence for ecosystem disruption (Kelly and Maguire 2009), impacts to food webs (DCR 2011), and potential to endanger Outstanding Ecoregions (ecoregions that are globally outstanding with regard to biodiversity) in California, Arizona, and Pennsylvania (Ricketts et al. 1999), provides justification for the economic costs of funding a biological control program for *N. peltata*.

Infested Area. Nymphoides peltata occurs in at least 27 states in all regions of the U.S. (Pfingsten et al. 2017). Of 239 records in EDDMaps, only 11 include information on infestation size. Of those, the average infestation size is 0.17 ha. However, because such a small number of reports included size, it is unclear what total area is infested in the U.S.

Expected Spread. The USDA conducted Weed Risk Assessments (WRA) for *N. peltata* in 2012 and concluded that the majority of the U.S. was suitable for establishment. *N. peltata* is already widely distributed, and the same report gives a high probability (>90%) of *N. peltata* becoming a serious invader (USDA 2012b) from current infestations.

As with *N. cristata*, a climate-matching study was conducted using known occurrences of *N. peltata* in its Asian range to predict climatic suitability of locations in the U.S. In comparison to locations where *N. peltata* is present, there is a large portion of the upper Midwest—particularly Iowa, Michigan, and Minnesota—that are vulnerable to establishment of new populations (Figure. 4). The southeastern states are less likely to have establishment of *N. peltata* based on climate matching, but Composite Match Index (CMI) values are still greater than 0.70, suggesting that climate is sufficient for plant establishment (Kriticos 2012).

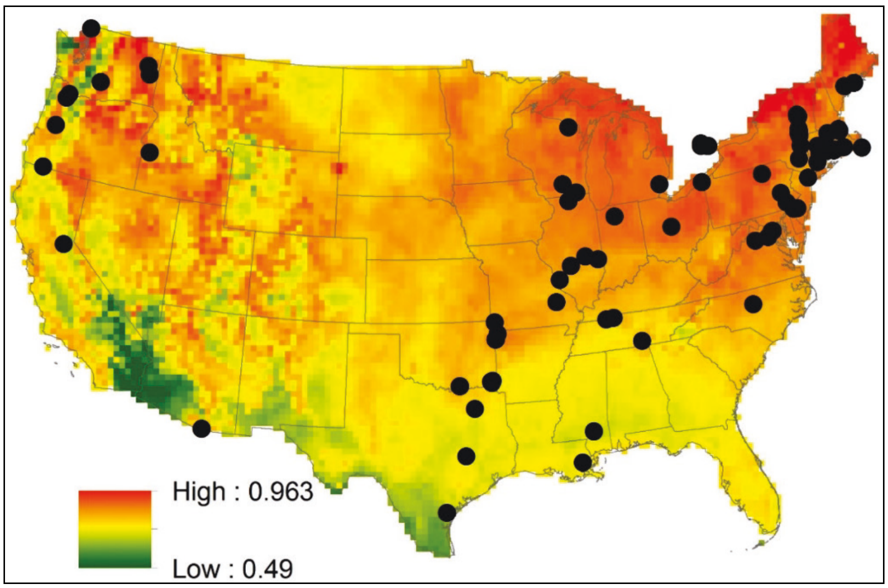

Figure 4. Results of climate-matching for *Nymphoides peltata*. Worldwide locations (excluding U.S. populations) were used in the model to identify areas in the U.S. with high suitability for survival. The majority of the U.S. is suitable for *N. peltata*. Black dots represent known occurrences from EddMaps. org.

Toxicity. This plant is apparently non-toxic to wildlife (Burrows and Tyrl 2012).

Available means of control

<u>Mechanical</u>. Mechanical harvesting is expensive and ineffective (Gren et al. 2007). Fragments produced during the mechanical harvesting process can readily persist and sustain the infestation (Nault and Mikulyuk 2013), roots and rhizomes are also able to withstand mechanical harvesting (Josefsson and Andersson 2001), and infestations have been observed to worsen following mechanical harvesting (Larson 2007a). In Willow Sump, Oregon, hand-pulling by local crews provided almost no control despite an expenditure of 800 person-hours over three weeks. Benthic

barriers were successful at eradicating a population at one site but the complexity of the habitat precluded their use on the larger infestation (Carri Pirosko, personal communication[1]).

Biological control. There are currently no known biocontrol agents for *N. peltata* (Nault and Mikulyuk 2013).

Herbicides. There is a paucity of relevant, contemporary research with regard to effective herbicide treatments of *N. peltata*. A report from Vermont, published in 1970, cites success with 2,4-D (Countryman 1970). More recent information suggests that Imazapyr and Imazamox were effective at controlling *N. peltata* in private ponds in Oregon (Carri Pirosko, personal communication[2]).

Environmental damage caused by control methods. Same as for *N. cristata*.

Economic justification of control methods. Mechanical harvesting of *N. peltata* is likely to be cost-prohibitive. A report from Sweden estimated costs at $9,000 U.S. per hectare to mechanically remove *N. peltata* (Gren et al. 2007). It is difficult to comment on the costs of herbicide management of *N. peltata*, as current cost estimates for long-term management of the plant are currently not available and can vary based on size of infestation and success rate of available herbicide formulations. In general, herbicides are moderately affordable within North America.

Beneficial aspects. Within North America there are five native *Nymphoides* that occupy a similar ecological niche as *N. peltata*. These species occupy aquatic habitats spanning a large area: from the Caribbean islands, Guatemala, and Mexico to the eastern regions of the United States. The novel introduction and continued presence of *N. peltata* in North America does not provide any additional environmental benefits that are not already supplied by native *Nymphoides* species as well as other native aquatic macrophytes. In contrast, the invasion of *N. peltata* has been shown to reduce biodiversity; impact community structure (Van der Velde 1976, Kelly and Maguire 2009, DCR 2011, USDA 2012b); substantially reduce oxygen levels during senescence of large monotypic stands (Cazacu and Gache 2005); and disrupt lake-wide food webs and ecosystems (Kelly and Maguire 2009, DCR 2011). There are no known positive benefits from the invasion of *N. peltata* and therefore there are no conflicts of interest to initiating a biocontrol program to manage this weed.

Infraspecific variation. Nymphoides peltata reproduces via vegetative and sexual means. Through vegetative means, it can form a new plant from rhizomes, stolons, separated leaves, or daughter plants (Nault and Mikulyuk 2013). Stolon production has been cited as the means for *N. peltata* to quickly colonize large areas in one growing season (Brock et al. 1983) and a single plant fragment has been attributed to large-scale waterbody colonization within a few years (Kelly and Maguire 2009). Zhonghua et al. (2007) documented the production of approximately 100 ramets per plant over a period of 12 weeks. *Nymphoides peltata* also reproduces readily through sexual means. Seed production is prolific, with estimates of between 3,000 to 9,000 seeds produced per square meter (Van der Velde and Van der Heijden 1981) and—while the literature is currently confounded on this topic—it appears that the plant is self-compatible (Ornduff 1970, Van der Velde and Van der

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[1] Pirosko, Cari. 2018. Personal communication with Nathan Harms via telephone. March 5.
[2] Ibid.

Heijden 1981) though self-pollinated seedlings may be less viable than those produced by out-crossing (Larson 2007b). Seeds are not negatively impacted by desiccation (Smits et al. 1989).

Native range. Nymphoides peltata is native to Eurasia. While quite abundant in Europe, it is not clear whether the plant is truly native or naturalized. In Sweden, at least, it is known to be introduced and is considered to be one of the worst invasive threats in the country (Larson 2007b). In contrast, it is widely accepted that Asia is part of the native range.

Relative abundance. Nymphoides peltata is listed as a noxious weed in many areas outside of its native range, including New Zealand (currently eradicated); Sweden (Larson and Willén 2006); Ireland (Kelly and Maguire 2009); and at least six states within the United States (NRCS 2017). However, in parts of its native range it is considered imperiled, leading to extensive restoration efforts to maintain populations (Nishihiro et al. 2009). The invasive potential of *N. peltata* has been well-documented and, similar to *N. cristata*, is due to its varied means of reproduction (see infraspecific variation section above) coupled with a strong competitive ability.

Nymphoides peltata is a good competitor with the ability to modify resource allocation as a means of survival. When grown together with two North American invasive species, the submersed *Myriophyllum spicatum* and floating-leaved *Trapa* sp., *N. peltata* allocated biomass to above-ground plant parts but overall growth was not significantly affected, indicating that it was able to tolerate both species (Wu and Yu 2004). Alternatively, an emergent plant, *Zizania latifolia*, was able to suppress *N. peltata* growth, flowering, and fruit production (Wu and Yu 2004). While *N. peltata* was negatively impacted by competition in that trial, it successfully reallocated 54% of biomass production to roots and stolons (Wu and Yu 2004) as a means of vegetative spread. In additional competition trials with North American native species (*Elodea canadensis*, *Ceratophyllum demersum*, and *Ranunculus circinatus*), *N. peltata* presence reduced the growth rate of all native plants at coverage levels as low as 33% *N. peltata* (Larson and Willén 2006).

Success of biological control elsewhere. Biological control of *Nymphoides peltata* has not been attempted elsewhere.

Number of known promising control agents. There are no known potential biological control agents for *N. peltata*, though at least four moths have been reported to feed on *Nymphoides aquatica* in the southeastern U.S.: *Langessa nomophilalis* Dyar, *Parapoynx allionealis* Walker, *P. seminealis* Walker, and *Synclita obliteralis* Walker (Herlong 1979, Stoops et al. 1998). Van Der Velde (1979) and Van der Velde et al. (1982) reported at least two lepidopterans (*Nymphula nymphaeata* (L.) and *Cataclysta lemnata* (L.)), a chironomid (*Cricotopus trifasciatus* (Mg.)), and several snails (*Lymnaea stagnalis* (L.), *Radix peregra* (Drap.), *Radix auricularia* (L.), *Galba turricula* (Held)) feeding on *N. peltata* in the Netherlands, but none of these reported species are likely host-specific and thus are of low value as potential control agents. In general, examples of herbivory or disease in the literature are rare. However, the fungal pathogen, *Septoria villarsiae*, is reported to produce leaf spots on *N. peltata* in a number of countries (Park et al. 2010) and should be examined further. In particular, domestic surveys may find *S. villarsiae* already present in the U.S., in which case it should be studied for use as a mycoherbicide.

Habitat stability. Biological control is likely to be most successful in areas with little disturbance (Peschken and McClay 1992). As with most aquatic weeds, however, infestations are likely to occur across a range of disturbance regimes. In general, the habitat occupied by *N. peltata* is likely to be stable, but disturbance from management activities (herbicides, mechanical removal) may be antagonistic if biological control agents are introduced. Additionally, shallow water conditions do not negatively affect the plant, as it is able to persist along muddy shorelines (Wu and Yu 2004).

Number of economic species in the genus Nymphoides. Same as for *N. cristata*.

Number of economic species in the family Menyanthaceae. Same as for *N. cristata*.

Number of ornamental species in the genus Nymphoides. Same as for *N. cristata*.

Number of ornamental species in the family Menyanthaceae. Same as for *N. cristata*.

Number of native North American species in the genus Nymphoides. Same as for *N. cristata*.

Number of North American species in the family Menyanthaceae. Same as for *N. cristata*.

Biological control Implementation. In order to initiate a biological control program for *Nymphoides* spp. in the U.S., two things must occur. First, the geographic source of introduced populations must be located (Goolsby et al. 2006). This is commonly done through molecular matching techniques in which plant tissues from introduced populations are sampled, their DNA is extracted and analyzed, then compared with samples from native populations (Williams et al. 2018). This approach is especially important when the species have large geographic ranges because control agents may be locally adapted to a particular plant genotype (Boughton and Pemberton 2011, Mukwevho et al. 2017). Second, searches can be focused to areas in which climate is similar to where populations occur in the introduced range. This climate-matching approach is geared towards finding agents that are pre-adapted to climatic conditions in the introduced range and have become commonplace with the advent of ecological modelling software. In order to locate genetic source populations, extensive sampling must occur in both native and introduced ranges. This should be a priority if a program is initiated to develop agents for the *Nymphoides*. However, climate-matching is already accessible and straightforward, so it is included in our assessment below.

The team used the ecological-modelling software package Climex (Hearne Ltd., South Yarra VIC, Australia) to employ regional climate-matching between introduced and native ranges of invasive *Nymphoides*. Maps generated from this process can be used to identify locations climatically similar to the invaded range in order to direct initial exploration for biological control agents. In addition to identifying areas in which climate should be suitable for *Nymphoides* growth and survival, natural enemies identified from searches in these locations should be pre-adapted to conditions in the U.S where infestations occur. First, the team accessed the EDDMapS (EDDMapS.org 2018) database to identify locations in the U.S. where the species have been documented. The team used these location points as reference locations in the Regional Climate Matching feature in Climex. Climate data used was at 10’ spatial resolution, averaged between 1961- 1990, downloaded from the CliMond database (Kriticos et al. 2012). Climex produces an index, from 0-1, of similarity between “home” and “away” locations, which is the Composite Match Index (CMI). This index is a combination of multiple climate variables, including maximum and minimum temperature, total rainfall, rainfall pattern, relative humidity, and soil moisture (Kriticos et al. 2015). For this analysis, only temperature and rainfall variables were used to determine similarity between locations. CMI outputs were imported into ESRI ArcMap (ver. 10.5; Redlands, CA) to create maps (Figures 5 and 6). Climate matching was accomplished separately for each species. Because *N. peltata* is native to Eurasia, the team assessed similarity between locations in both Europe and Asia. *Nymphoides cristata* is only reported from Asia, so examination was limited to that continent.

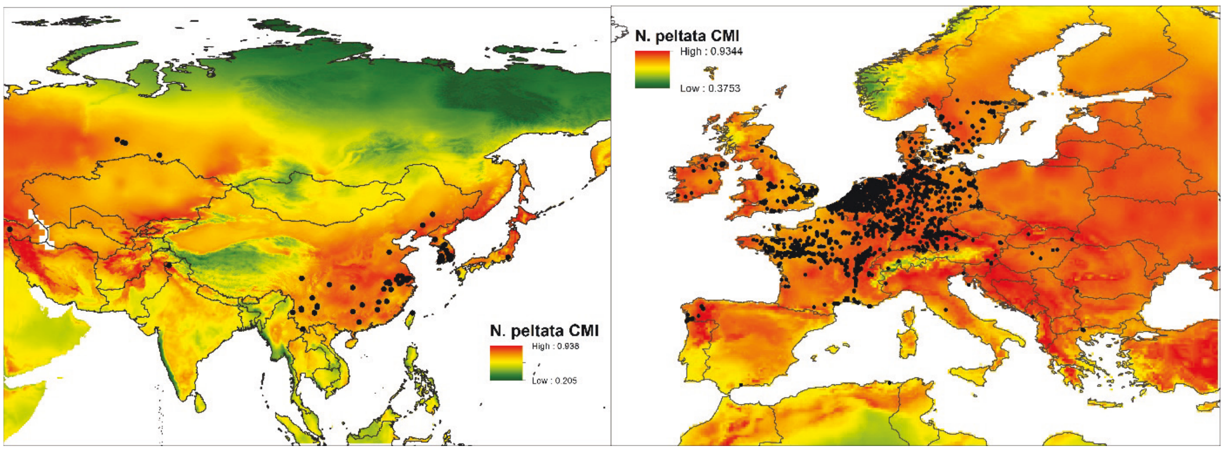

Figure 5. Results of climate-matching for *Nymphoides peltata* in Asia and Europe. Known occurrences in the U.S. were used for comparison between locations. Warm colors represent areas of closest climatic match to U.S. locations. Black dots represent records of *N. peltata* in each region.

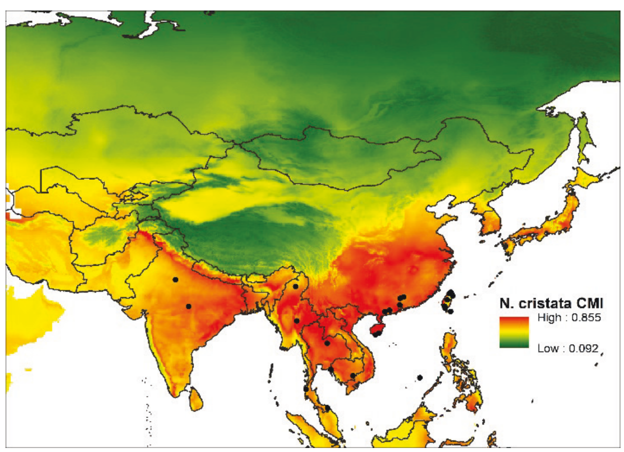

Figure 6. Results of climate matching for *Nymphoides cristata* in Asia. Known occurrences in the U.S. were used for comparison between locations. Warm colors represent areas of closest climatic match to U.S. locations. Black dots represent records of *N. cristata*.

Climate-matching for both *Nymphoides* spp. produced valuable information. For *N. peltata*, the team identified priority locations throughout nearly all of Europe, Eastern China, and the Korean peninsula (Figure 2). These locations match closely with areas where *N. peltata* has been previously recorded in the native range, suggesting agents discovered during surveys should be climatically adapted to locations in the U.S. *Nymphoides peltata* in the U.S. has a wide distribution, occurring in both southern and northern states; consequently, foreign surveys may benefit from covering a similarly large area. Additionally, as the genetic structure of *N. peltata* populations in the U.S. is characterized, foreign surveys can become more targeted and localized. *Nymphoides cristata* is regionally abundant in the southeastern U.S., and overseas surveys will benefit from focusing on areas of Southeast Asia (Figure 3). As with *N. peltata*, once genetic studies confirm source populations, searches for biological control agents can be more focused.

CONCLUSION: Based on review of the literature and calculation of the Peschkin-McClay score, the team concludes that both introduced *Nymphoides* are appropriate targets for biological control in the U.S. Vegetative and sexual reproduction in introduced *Nymphoides* will promote further spread beyond locations where they currently exist and evidence of strong competitive ability suggests many plant communities will be at risk of negative impacts from establishment of introduced *Nymphoides*. The majority of the southeastern U.S. is at risk from *N. cristata*, and a large portion of the Midwestern U.S. is at risk from *N. peltata*. Management options are currently limited. Mechanical removal is likely to be cost-prohibitive or may lead to further spread when plant material is fragmented during harvesting. Although herbicide research is ongoing for *N. cristata*, a means for determining the optimal treatment for *N. peltata* is lacking. Because biological control is a long-term investment, the authors recommend initiating a new program in the near-term, with examination of genetic structure of both species in the U.S., determination of potential hybridization in the U.S. between native and invasive *Nymphoides*, and determination of potential areas of source material in Europe and southeastern Asia. During collection of material for genetic analyses, preliminary surveys can be conducted to determine whether any agents are already present in the U.S. and to determine potential biological control agents in the native range.

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