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## **Surveys for Biological Control Agents of *Hydrilla verticillata* in the People's Republic of China in 2013**

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# **Surveys for Biological Control Agents of *Hydrilla verticillata* in the People's Republic of China in 2013**

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## Abstract

This report summarizes the result of 2013 surveys in the People's Republic of China of the invasive aquatic weed *Hydrilla verticillata* for potential insect biological control agents. Plants were examined at 34 sites throughout eastern China, resulting in the collection of leaf-mining *Hydrellia* spp. (Diptera: Ephydriidae), *Parapoynx diminutalis* (Lepidoptera: Crambidae), various Chironomidae (Diptera), *Macrolea japonica* (Coleoptera: Chrysomelidae), and other unidentified insect herbivores. Additionally, effort was made to identify plants as monoecious or dioecious, since both biotypes are present in the U.S. However, at the majority of sites only dioecious plants were apparent. Previous surveys in China were limited in scope and never included biotype identification; consequently, the present work represents an initial attempt to locate biotype-matched hydrilla biological control agents for use in the U.S. An Appendix with site locations and descriptions is included.

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## Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP). The APCRP is sponsored by Headquarters, U.S. Army Corps of Engineers (USACE), and is assigned the U.S. Army Engineer Research and Development Center (ERDC) under the purview of the Environmental Laboratory (EL). Funding for the APCRP was provided under the Department of the Army Appropriation Number 96x3122, Construction General. Dr. Linda S. Nelson was Program Manager for the APCRP.

The work was performed under the general supervision of Dr. Timothy Lewis, Chief of the Aquatic Ecology and Invasive Species Branch; Mark Farr, Chief of the Ecosystem Evaluation and Engineering Division; and Dr. Beth C. Fleming, Director of the EL.

At the time of publication of this report, COL Bryan S. Green was Commander of ERDC, and Dr. David W. Pittman was Director of ERDC.

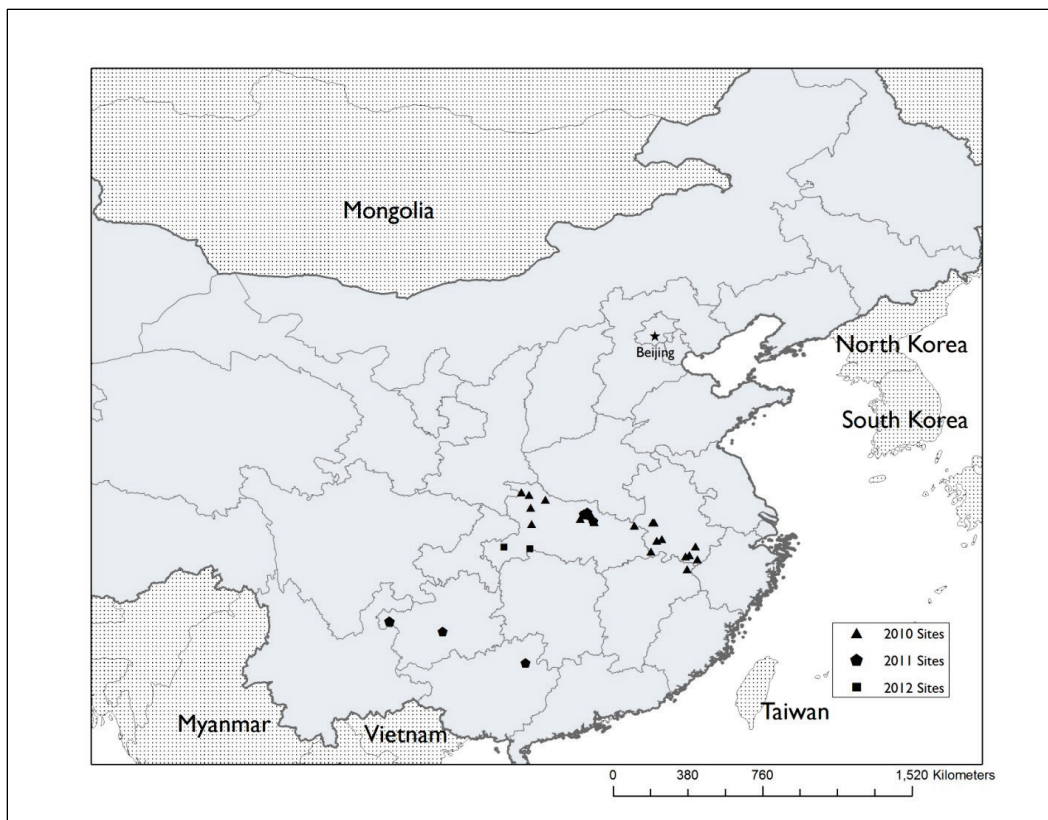
# 1 Introduction

## Background

Hydrilla is a submersed invasive plant native to Africa, Asia, Australia, and parts of Europe (Buckingham and Grodowitz 2004) and has been present in the U.S. since the 1950s (Schmitz et al. 1991). Two hydrilla biotypes are currently recognized in the United States: the dioecious biotype (male and female flowers located on separate plants; only female plants are found in the U.S.) is generally found in the southeastern and south central U.S., whereas the monoecious biotype (both male and female flowers found on the same plant) mostly occurs in the central Atlantic and northeastern U.S. (Madeira et al. 2000). Though the dioecous biotype has been present in the U.S. for nearly 60 years, monoecious hydrilla is widely recognized as a relatively recent introduction. It was first discovered in Delaware in 1976 (Steward et al. 1984) and has since been reported in Alabama, California, Connecticut, Georgia, Indiana, Kansas, Massachusetts, Maine, Maryland, Missouri, New York, North Carolina, Ohio and Washington ([www.northeastans.org](http://www.northeastans.org), Madeira et al. 2000, Maki and Galatowitsch 2008).

Hydrilla (presumably the dioecious biotype, since information regarding biotype was seldom collected) has been surveyed for biological control agents in various parts of its native range since the 1960s (Balciunas et al. 2002), which led to the testing and release of two ephydrid flies (*Hydrellia pakistanae* Deonier and *H. balciunasi* Bock) and two curculionid beetles (*Bagous hydrillae* O'Brien and *B. affinis* Hustache) in the U.S. Only *H. pakistanae* and *H. balciunasi* established and dispersed, with *H. pakistanae* being the most wide spread and commonly encountered agent (Grodowitz et al. 2004). More recently, a collaborative effort between the US Army Corps of Engineers (USACE), the US Department of Agriculture Agricultural Research Service (USDA-ARS), the Australian Biocontrol Laboratory (ABCL), the *Commonwealth Scientific and Industrial Research Organization* (CSIRO), and the Chinese Academy of Sciences (CAS) has led to the examination of hydrilla throughout southern and central PRC (Ding et al. 2011, Zhang et al. 2012; Figure 1). These surveys have focused on geographic locations widely considered to be the epicenter of hydrilla evolution (Cook and Luond 1982, Overholt et al. 2008) and the likely location for discovering effective biocontrol agents against the dioecious strain currently established in the U.S. (Madeira et al. 1999); geographically matching herbivores and host plants may increase likelihood of a successful biocontrol program (e.g., Goolsby et al. 2006, Manrique et al. 2008).

Figure 1. Hydrilla survey sites in the People's Republic of China from 2010-2012.



As a result, a number of potential biocontrol agents, including several *Hydrellia* spp. and *Bagous* spp., have been collected over the last several years and colonies have been established in CAS and ABCL laboratories to determine host-specificity and develop more effective rearing methods (Ding et al. 2011, Zhang et al. 2012).

## Objective

With the more recent establishment and rapid spread of monoecious hydrilla in the US, it has been recognized that research efforts should be focused on determining whether the currently available suite of biocontrol agents are suitable to manage that biotype (Grodowitz et al. 2010). Mixed results have been reported (Dray and Center 1996, Grodowitz et al. 2010), but the differences in growth and phenology between the two hydrilla biotypes could partly explain why the introduced *Hydrellia* spp. may not persist on monoecious hydrilla (Harms and Grodowitz 2011). Additionally, it is unclear whether original surveys for biocontrol agents of hydrilla examined monoecious or dioecious plants. Buckingham (1998) suggested that additional natural enemy surveys of more temperate hydrilla strains

were needed. For these reasons, it is important to expand the ongoing biocontrol agent surveys on the dioecious biotype while also targeting monoecious plants in their native range. This is likely the best chance for identifying and eventually testing agents that are better suited for the dioecious or monoecious biotype.

## **Approach**

The main focus of this technical report is to:

- Detail monoecious and dioecious hydrilla biocontrol agent surveys in PRC and, to a minimal extent, in southeastern PRC between May and October 2013

## 2 Materials and Methods

### Surveys for Herbivore Species

Prior to travel in PRC, sites were identified through examination of herbarium specimens, online database searches (<http://www.cvh.org.cn/>), and contacts with PRC scientists. Other site visits were unplanned; if during transit from one sampling location to another surfaced aquatic vegetation was observed in a waterbody, then the site was examined for presence of hydrilla. Several sites were inaccessible due to record flooding in the far northeastern areas of the country, thus flexibility in travel was required. Site types were diverse and included urban and rural ponds/lakes and rivers, man-made canals, shallow irrigation ditches and natural wetlands.

Plants were collected one of several ways: hand collection from shore by tossing a double-sided metal rake with a rope handle and snagging plants during the retrieval, hand collection by wading, and collection from a boat by hand or using a rake. Plants were visually examined in the field for 5-15 minutes for signs of herbivory. Approximately 1 kg of plant material was collected from each site, placed in a zip lock plastic bag, and sealed until further examination. In addition, plants were examined for flowers to determine biotype.

Table 1 lists sites where hydrilla was examined during the 2013 survey. Appendix A contains a summary of site information and photographs.

### Processing Samples, Identification, and Rearing of Herbivore Species

In general, processing of the collected hydrilla occurred in hotel rooms. Rooms were reserved with the prerequisite they have access to refrigeration in order to keep plants from degrading. In southern regions, a portable refrigerator was also used when travelling to preserve hydrilla samples. One sub-sample from each collection was initially hand examined with the aid of a jeweler's loop (10X magnification) and any herbivores or feeding damage was recorded. The remaining material was placed into a portable, cloth Berlese funnel utilizing a single 40 watt bulb to progressively dry the hydrilla material and drive internally/externally feeding herbivores into a collection vessel containing water. These vessels were inspected daily until the plant material in the Berlese was completely dry. Using a fine brush,

Berlese-extracted aquatic moth larvae were carefully transferred to plastic rearing containers (75x75x50 mm) which contained ~5cm stem fragments of hydrilla and ~1cm of water. Other herbivorous insect larvae were either too difficult to rear while travelling (e.g., Chironomidae require aeration) or adults were reared from the same material (e.g., *Hydrellia* flies). Adults and immatures of other herbivores were preserved in 95% ethanol (EtOH) as voucher specimens for later identification.



Table 1. Locations where hydrilla was surveyed and examined in 2013. “Damage” refers to whether herbivore damage was observed on plants during examination. Hydrilla biotype was determined in the field based on flowering plants, but plant samples were also collected for later genetic verification. “dio”= dioecious, “mono” = monoecious, “NF” = not flowering

State/Province	Site	Date	GPS Location		Hydrilla biotype	Herbivore	Number/Stage		
							Adults	Larvae	Damage
Anhui	Tianjinghu Lake	22 May 2013	N 30° 56.97'	E 117° 47.60'	NF	None			
	Gaoyuan Village	23 May 2013	N 30° 24.90'	E 117° 46.69'	NF	Chironomidae		6	
						<i>Parapoynx</i> sp.		4	
Beijing	Kunming Lake (Summer Palace)	12 August 2013	N 39° 59.66'	E 116° 15.67'	Dio	<i>Parapoynx</i> sp.		7	
	Cuihu Wetland Park	12 August 2013	N 40° 06.24'	E 116° 10.45'	Dio	None			
	Zizhuyuan Park (Royal Bamboo Gardens)	13 August 2013	N 39° 56.53'	E 116° 18.78'	Dio	<i>Hydrellia</i> sp.			Y
	Yuyuantan Park (August 1st Lake)	13 August 2013	N 39° 55.01'	E 116° 18.51'	Dio	None			
	Yanqi Lake	16 August 2013	N 40° 23.67'	E 116° 40.47'	NF	None			
	Yanqi River	16 August 2013	N 40° 22.06'	E 116° 40.19'	NF	None			
	Chaobaihe River	17 August 2013	N 40° 22.93'	E 116° 53.45'	Dio	None			
Guangxi	Shiwaitaoyuan Park	27 September 2013	N 24° 52.02'	E 110° 23.05'	Dio	<i>Macroplea japonica</i>		2	
Hebei	Baiyangdian Wetland	14 August 2013	N 38° 56.57'	E 115° 57.72'	Dio	<i>Parapoynx</i> sp.		8	
	Zhainan Village	14 August 2013	N 38° 53.98'	E 115° 58.47'	Dio	<i>Parapoynx</i> sp.		12	
	Wulie River, Chengde	17 August 2013	N 40° 57.67'	E 117° 56.55'	Dio	None			

State/Province	Site	Date	GPS Location		Hydrilla biotype	Herbivore	Number/Stage		
							Adults	Larvae	Damage
	Bishu shan zhuang, Chengde	17 August 2013	N 40° 58.95'	E 117° 56.32'	Dio	None			
Heilongjiang	Harbin Fun Park	28 August 2013	N 45° 46.82'	E 126° 39.79'	Dio	Chironomidae		4	
						<i>Hydrellia</i> sp.		3	
						<i>Macrolea japonica</i>		2	
Henan	Guangshan County	4 July 2013	N 32° 00.55'	E 114° 55.17'	NF	None			
	Changheqiao Village	4 July 2013	N 31° 41.58'	E 115° 00.82'	NF	Chironomidae		9	
						Elmidae	2	3	
	Jianhechang Town	4 July 2013	N 31° 31.58'	E 114° 48.48'	NF	Chironomidae		23	
	Lijiazhai Town	4 July 2013	N 31° 53.72'	E 114° 05.53'	NF	Chironomidae		7	
						<i>Hydrellia</i> sp.	4		
Hubei	Lianglukou Village	3 July 2013	N 31° 22.96'	E 115° 09.20'	NF	Chironomidae		6	
						<i>Hydrellia</i> sp.	7		
						Trichoptera	2		
Hunan	Dingjiashan Village	13 September 2013	N 29° 36.92'	E 111° 28.98'	Mono	<i>Hydrellia</i> sp.	2	2	
	Wuxi Village	14 September 2013	N 29° 04.45'	E 110° 21.52'	NF	<i>Hydrellia</i> sp.		2	
						<i>Parapoynx</i> sp.		17	
	Pengjiazhaishang Village	14 September 2013	N 28° 55.52'	E 110° 00.89'	Mono & Dio	Chironomidae		9	
	Gaoping Town	14 September 2013	N 28° 50.78'	E 109° 58.63'	NF	None			
	Tuxipu Village	15 September 2013	N 26° 46.55'	E 109° 42.25'	Mono	Chironomidae		11	
						<i>Hydrellia</i> sp.		2	

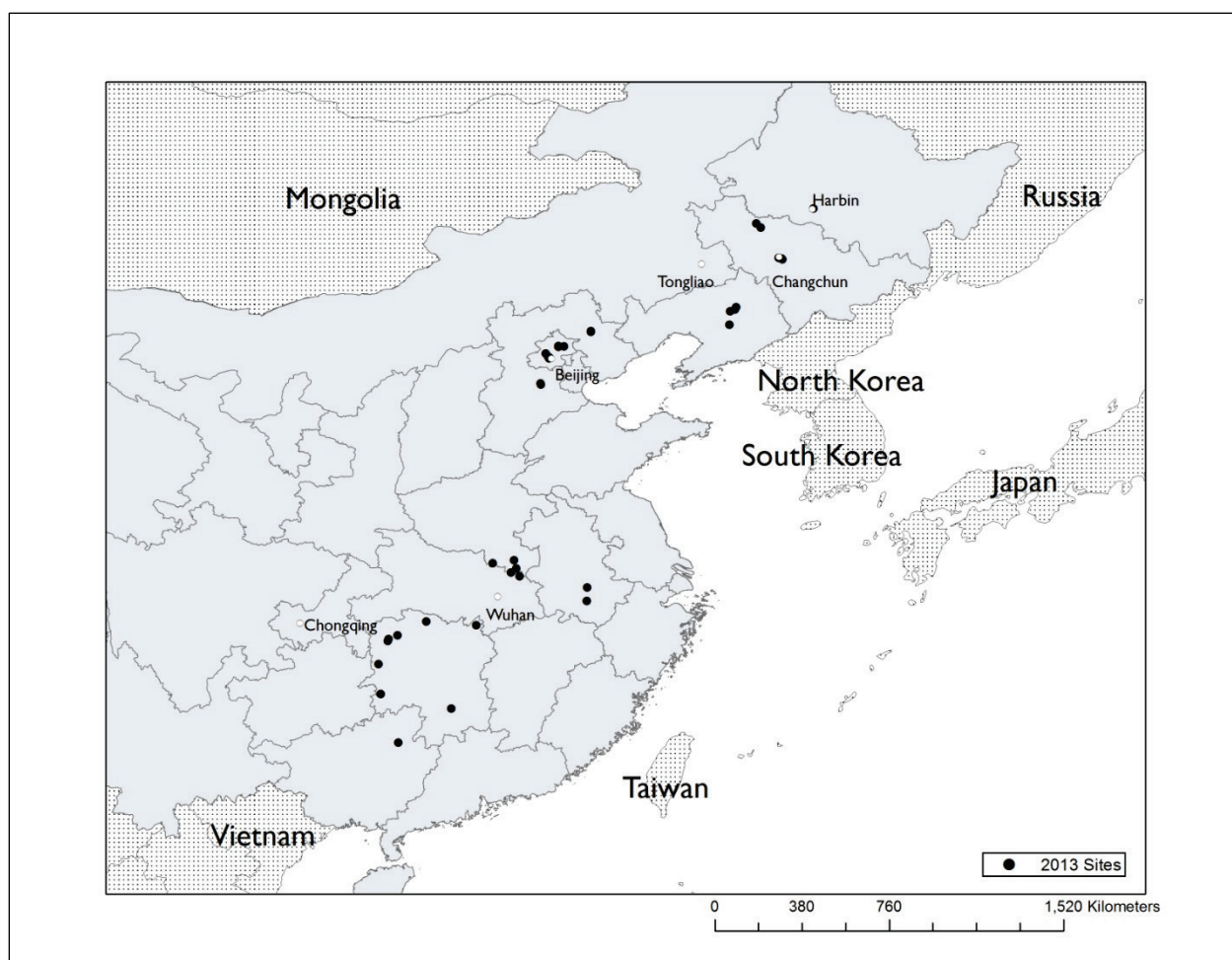
State/Province	Site	Date	GPS Location		Hydrilla biotype	Herbivore	Number/Stage		
							Adults	Larvae	Damage
Jilin	Nan hu Lake, Changchun	19 August 2013	N 43° 51.06'	E 125° 17.96'	Dio	<i>Parapoynx</i> sp.		1	
	Boxue Road, Changhun	19 August 2013	N 43° 49.25'	E 125° 25.63'	Dio	None			
	Jingyue Lake Park, Changchun	19 August 2013	N 43° 47.76'	E 125° 26.77'	Dio	<i>Hydrellia</i> sp.		17	Y
						<i>Parapoynx</i> sp.		7	
	Highway G203	24 August 2013	N 45° 02.70'	E 124° 36.51'	Dio	Chironomidae		6	
						<i>Parapoynx</i> sp.		2	
	Chagan hu Lake	25 August 2013	N 45° 12.22'	E 124° 25.63'	Dio	<i>Hydrellia</i> sp.		9	
Liaoning	Puhe River, Qipanshan Lake, Huishan Village	20 August 2013	N 41° 54.91'	E 123° 38.51'	Dio	<i>Hydrellia</i> sp.		26	Y
	Nan hu Park, Shenyang	21 August 2013	N 41° 46.03'	E 123° 24.06'	Dio	None			
	Dong hu Lake, Shenyang	21 August 2013	N 41° 49.64'	E 123° 35.66'	Dio	<i>Hydrellia</i> sp.		25	Y
						Chironomidae			Y
	Tang River, Qijiattun Village	22 August 2013	N 41° 13.80	E 123° 22.34	Dio	Chironomidae		3	

### 3 Results and Discussion

#### Surveys for Herbivore Species

A total of 66 sites were visited during 2013 surveys in the PRC. Hydrilla was observed at 52% of the sites and examined (Table 1, Figure 2) for herbivores. Hydrilla was not located at 48% of the surveyed sites.

Figure 2. 2013 hydrilla herbivore sampling locations in Peoples Republic of China.



Hydrilla could not be found at many sites due to flooding. However, in other areas it is possible that changes in soil substrate or water composition may have been responsible for changes in plant species structure over large areas. Most notably, no hydrilla could be found travelling from Shenyang to Tongliao in Inner Mongolia, in and around Tongliao, and travelling to the border of Inner Mongolia on route to

Songyuan. Similar results were obtained travelling from Songyuan to Daqing, Duer Bote County south of Daqing, and travelling from Daqing to Harbin. In these areas we also noted an absence of lotus plants, *Nelumbo nucifera*, which had been present in many of the other areas that hydrilla was collected during 2013 surveys.

## Identification and Rearing of Herbivore Species

The most common herbivores extracted from collections in PRC were larvae of the leaf-mining fly, *Hydrellia* sp., which were found, or associated-damage was observed, at 11 sites in the northeastern and southern regions. Eighty-six larvae were extracted from samples processed in Berlese funnels from eight sites and at three sites a total of 13 adults were collected. Of interest, *Hydrellia* sp. adults and larvae were collected from monoecious hydrilla at Dingjiashan Village in Hunan Province. Adults have been forwarded for identification to Dr. John Deeming (National Museum of Wales) who is currently reviewing *Hydrellia* spp.

Aquatic moth larvae were extracted from hydrilla samples from eight sites. No adults were collected from Berlese funnels or subsequently reared. All larvae were *Parapoinx* sp. *prob. diminutalis* (Lepidoptera: Pyralidae) which is already present in the United States. Adult wasps (Hymenoptera) from the sample collected at Zhainan Village in Hebei Province were possibly parasites of the *Parapoinx* larvae given they were the only herbivores extracted from the sample.

Seventy-eight chironomid larvae were extracted from samples collected at 10 sites. These midges usually attack the apical meristems of hydrilla where feeding damage is typically minimal (Purcell, personal observation). Damage only was observed at Dong Hu Lake in Liaoning Province which consisted of burrows or retreats formed in stems between internodes within 10 cm of the growing tip.

Larvae of *Macrolea japonica* (Chrysomelidae: Donaciinae), which feed on the roots of hydrilla, were collected from Shiwaitaoyuan Park in Guangxi (two larvae) and from Harbin Fun Park in Heilongjiang (2 larvae). This is the only species of *Macrolea* that we have collected in widespread surveys across southern, central and northern China. Previous evaluations determined that this beetle is non-specific, feeding on a variety of aquatic plants, including *Vallisneria* sp. and *Potamogeton* spp. among others (Zhang et al. 2010). Adults and larval riffle beetles (Coleoptera: Elmidae)

were collected in Henan Province but these beetles do not appear to damage hydrilla. Two adult caddisflies (Trichoptera) were also extracted from Berlese funnels from a sample collected in Hubei Province but their herbivorous larvae were not found. These larvae usually reside in cases made from plant material or from other substrates within which they pupate. We could not confirm if these adults emerged from immatures that fed on hydrilla.

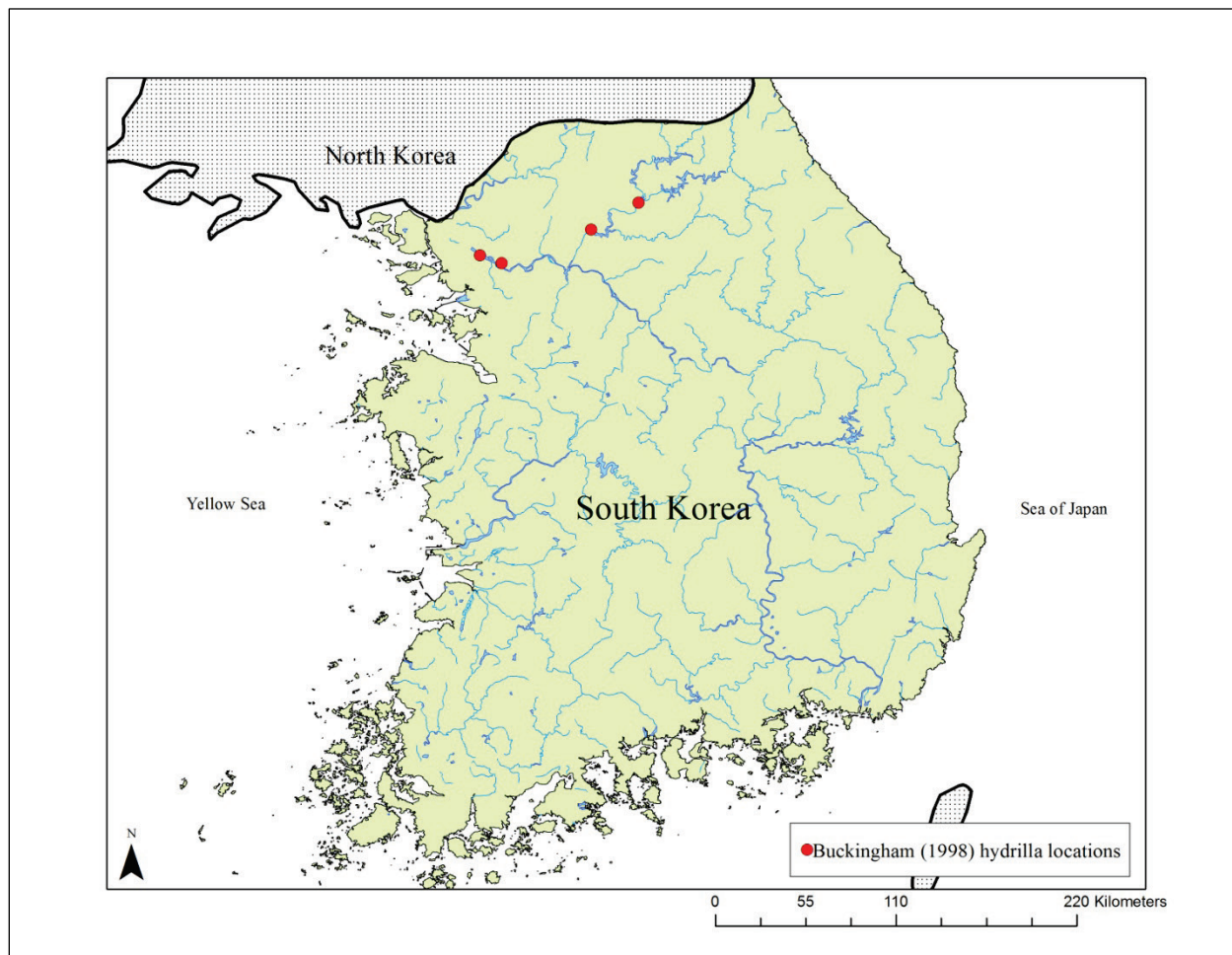
No *Bagous* weevils were collected in China during 2013. A colony of *Bagous rufipennis* is still being maintained at ABCL quarantine in Brisbane. These insects were originally collected from Hubei Province in 2011 and the progeny were used in host range testing in 2012. Further testing could not be completed during 2013 as a disease outbreak drastically reduced adult numbers in the colony. Since the decline, adult numbers have slowly increased and testing will resume in 2014. A small number of *Bagous* sp., currently unidentified but referenced as *Bagous* sp. 2, were also imported into ABCL quarantine in November 2012 and maintained for more than 6 months. During this period adults fed but failed to oviposit and were subsequently preserved. More research is needed on *Bagous* sp. 2 in China given that this species was extracted from field collected hydrilla and is, so far, the only weevil collected from hydrilla that appears to complete development on the plant under water.

Monoecious hydrilla has previously been collected at numerous sites in the PRC (Steward 1993), including areas near Beijing where a portion of the 2013 study was centered. Despite previous accounts, monoecious plants were not visually identified from any of the sites in northeastern China (Table 1). Plants were collected which showed no signs of flowers (male or female), including several that appeared morphologically similar in general appearance to monoecious plants found in the US. Samples of monoecious hydrilla were collected and examined for herbivores in southern China at Dingjiashan Village, Tuxipu Village, Yaolaitan Hydropower Station and possibly Pengjiazhaishang Village in Hunan Province. Since it was not possible to determine biotype of non-flowering plants in the field, a concurrent study is examining the plant material collected for genetic characterization to determine biotype (monoecious or dioecious) and genetic similarity to US hydrilla plants.

## Future Directions

The source of US monoecious plants is still unclear, but the general consensus is that they are genetically most similar to Korean accessions (Madeira et al. 2007, Benoit 2011). Therefore, the logical progression of this project is to collect plants from sites in South Korea. Based on limited South Korean surveys in the 1990s, there are site descriptions available which will aid in locating both dioecious and monoecious plants (Buckingham 1998). Previous surveys were very limited geographically (Figure 3); thus, more widespread examination is needed. Potential collaborators in South Korea have been identified to assist in logistical planning and collections in 2014. In addition, sites that were flooded during the current survey in northeastern PRC should be revisited and sampled for agents and plant samples collected for genetic characterization (Figure 2).

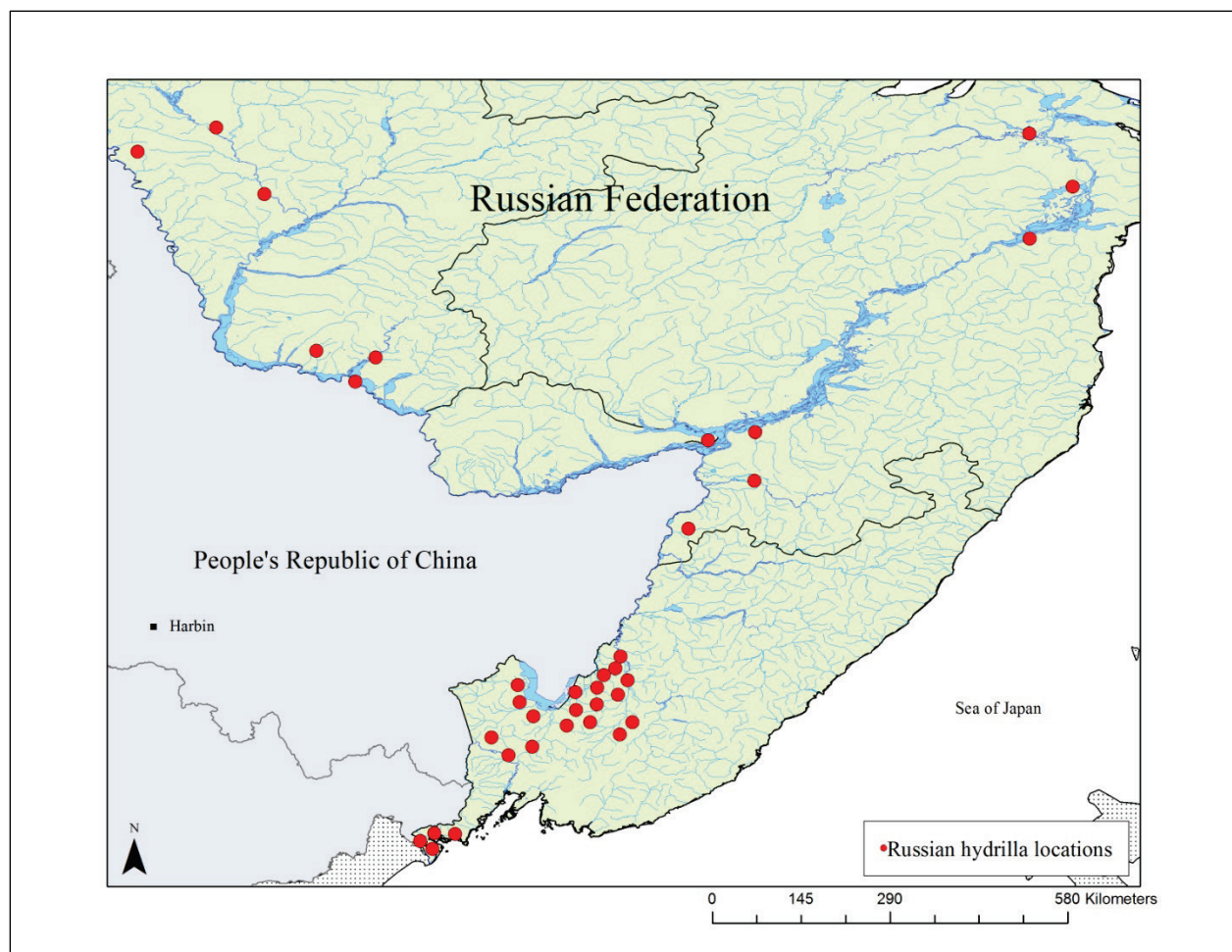
Figure 3. Approximate hydrilla collection sites reported by Buckingham (1998) during the only Korean survey conducted.





Hydrilla has been reported from far-eastern Russia (Figure 4), including areas near the border with the PRC (Probatova and Buch 1981, Zaitzev et al. 1996). Surveys for biocontrol agents have never taken place in these areas despite predictions that potential agents may be identified (Zaitzev et al. 1996). It is important that collections take place in these northern areas that may identify agents more suited to northern climates in the US, in addition to providing a more complete picture of the native range of monoecious and dioecious biotypes.

Figure 4. Russian hydrilla collection sites from Probatova and Buch (1981).





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## Appendix A: Sites Visited

**Site:** Tianjinghu Lake, Anhui Province (N 30°56.97 E 117°47.60')



**Date:** 22 May 2013

**Species present:** *Hydrilla*, *Alternanthera philoxeroides*, *Potamogeton* sp.

**General information:** A large clear lake with road access within. The lake was very deep with few aquatic plants present. Several scattered hydrilla plants and small mats were observed along the shoreline. No flowers were observed on hydrilla and no insects were collected from hydrilla samples.

**Site:** Gaoyuan Village, Anhui Province (N 30°24.90', E 117°46.69')



**Date:** 23 May 2013

**Species present:** Hydrilla, *Potamogeton* sp., Poaceae sp.

**General information:** A river site with an irrigation channel running parallel. Hydrilla formed large submerged mats on the substrate, and an unknown grass species emerged at the water surface. In the canal, *Potamogeton* sp. plants grew around the margins of the hydrilla mats. No flowers were observed on hydrilla plants. Chironomids and foliage feeding *Paraponyx* sp. larvae found in sample.



**Site:** Lianglukou Village, Hubei Province (N 31°22.96', E 115°09.20')



**Date:** 3 July 2013

**Species present:** Hydrilla, *Potamogeton* sp., *Myriophyllum* sp.

**General information:** Hydrilla was collected from a river at the base of a hill. The water was clear, but there was evidence of sediment accumulation on the aquatic plant leaves. Hydrilla formed monospecific stands. *Hydrellia* sp. and chironomids were observed on hydrilla plants at this site. No flowers were observed on hydrilla.

**Site:** Guangshan County, Henan Province (N 32°00.55', E 114°55.17')

**(No photograph available)**

**Date:** 4 July 2013

**Species present:** Hydrilla

**General information:** This site is a drainage ditch in the front of the County Government Office Buildings of Guangshan. Large amounts of hydrilla plants were scattered on the muddy substrate; no insects were found. Hydrilla was very robust; there were no other aquatic plants at this site. No flowers were observed on hydrilla plants.

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**Site:** Changheqiao Village, Henan Province (N 31°41.58', E 115°00.82')

**(No photograph available)**

**Date:** 4 July 2013

**Species present:** Hydrilla, *Potamogeton* sp.

**General information:** This site is a shallow river. Boulders emerged from the water between which hydrilla and *Potamogeton* sp. plants were dispersed. No flowers were observed on hydrilla plants.

**Site:** Jianhechang Town, Henan Province (N 31°31.58', E 114°48.48')



**Date:** 4 July 2013

Species present: Hydrilla

**General information:** This site is a river with muddy substrate. Several small patches of hydrilla were scattered near the shore. The hydrilla plant material including the roots was covered in snails. No flowers were observed on hydrilla plants.



**Site:** Lijiazhai Town, Henan Province (N 31°53.72', E 114°05.53')

**(No photograph available)**

**Date:** 4 July 2013

**Species present:** Hydrilla, *Potamogeton* sp.

**General information:** This site is a deep river near the gate of Lijiazhai Town. A few hydrilla plants were scattered along the bottom with *Potamogeton* sp. No flowers were observed on hydrilla plants.

**Site:** Kunming Lake, Summer Palace Pond, Beijing, China (N 39° 59.66', E 116° 15.67')



**Date:** 12 August, 2013

**Species present:** *Hydrilla*, *Nelumbo nucifera*, *Myriophyllum spicatum*, *Potamogeton malaianus*, *Potamogeton perfoliatus*, *Potamogeton* sp.

**General information:** A small tourist boat was used to examine the main lake. The boat was parked on the far shore and an adjacent wetland (~50 m from the boat) was examined. The wetland was approximately 100 m across. Although no hydrilla was observed in the main lake, patches were found in the wetland. Hydrilla in the wetland grew in clumps with native vegetation, including *Myriophyllum*. Hydrilla made up a small proportion (~5%) of the total vegetation, which covered ~30% of the water body. Both male and female hydrilla plants were observed (dioecious), and no monoecious hydrilla plants were obvious. Hydrilla samples were taken 1-2 m from shore at a point where water depth was 1.5-2 m. Substrate was clay.

**Site:** Cuihu Wetland Park, Beijing, China (N 40° 06.24', E 116° 10.45')



**Date:** 12 August, 2013

**Species present:** *Hydrilla*, *Chara* sp., *Nelumbo nucifera*, *Potamogeton malaianus*, *Typha* sp., *Vallisneria* sp. (prob. *spiralis*)

**General information:** Cuihu Wetland Park is a constructed wetland project in Beijing. The wetland was still under construction but access was provided by a biologist. *Hydrilla* was scarce in the park. Several *hydrilla* plants had up to eight leaves per whorl. Male plants were observed. Aquatic vegetation covered approximately 20% of the water's surface, and *hydrilla* made up ~20% of aquatic plant cover. *Hydrilla* samples were taken 0.5-1 m from shore at a point where water depth was 0.5-1 m. Substrate was silt/clay.



**Site:** Zizhuyuan Park (Royal Bamboo Gardens), Beijing, China (N 39° 56.53', E 116° 18.78')



**Date:** 13 August, 2013

**Species present:** *Hydrilla*, *Chara* sp., *Nelumbo nucifera*, *Potamogeton* sp., *Sagittaria trifolia*, *Vallisneria* sp.

**General information:** Royal Bamboo Park is a series of connected ponds and canals in Beijing. A floating fragment of hydrilla was spotted in a canal and a stand of hydrilla was found in an adjacent pond. The pond was very shallow (0.6 m depth), approximately 50 m in diameter, and nearly 80% covered in aquatic vegetation, 10% of which was hydrilla. Hydrilla was abundant in clumps along the pond margin. *Hydrellia* sp. flies were observed on lotus leaves. Though not measured, water clarity was high relative to the water in the adjacent canal. Both male and female plants were collected, but no monoecious plants were identified. Hydrilla samples were collected 1-2 m from shore at a point where water depth was ~0.6 m. Substrate was silt/ clay. *Sagittaria trifolia* plants that were heavily infested with an unidentified stem-boring caterpillar were also present at this site.

**Site:** Yuyuantan Park, August 1st Lake, Beijing, China (N 39° 55.01', E 116° 18.51')



**Date:** 13 August, 2013

**Species present:** *Hydrilla*, *Ceratophyllum demersum*, *Nelumbo nucifera*, *Najas* sp., *Nymphaea* sp., *Potamogeton malaianus*.

**General information:** August 1<sup>st</sup> Lake is located in Beijing and has been visited by previous biocontrol scientists (Buckingham 1998) many times. *Hydrilla* was found during the current survey in an adjacent wetland separated from the main lake by a short canal. Aquatic vegetation covered ~ 50% of the wetland, while *hydrilla* made up ~20% of total aquatic vegetation. Both male and female plants were collected, though no plants could be visually identified as the monoecious biotype. *Hydrilla* samples were collected 0.5 m from shore at a point where water depth was 1 m. Substrate was silt/ clay.



**Site:** Baiyangdian Wetland, Hebei, China (N 38° 56.57', E 115° 57.72')



**Date:** 14 August, 2013

**Species present:** *Hydrilla*, *Nymphaea* sp., *Potamogeton malaianus*, *Potamogeton* spp., *Salvinia* sp.

**General information:** *Hydrilla* was only found near the park entrance to the lake. *Hydrilla* was rare at this site and only male plants were collected at 0.5-1 m from shore and a depth of 0.5-1m. The lake/wetlands were explored by boat for ~2 hours but no additional *hydrilla* plants were found. *Ceratophyllum* sp., *Myriophyllum* sp. and *Utricularia* sp. were common, and approximately 50% of the water's surface was covered in aquatic vegetation. Substrate was clay.

**Site:** Zhai nan village, Hebei, China (N 38° 53.98', E 116° 18.78')



**Date:** 14 August, 2013

**Species present:** *Hydrilla*, *Ceratophyllum* sp., *Potamogeton crispus*.

**General information:** Contact was made with a local aquarium plant merchant and a meeting was arranged on the street in Zhai nan village. A bag of mixed plant species, predominantly hydrilla, was purchased from the merchant. Only female flowering plants were observed in the bag. The exact location of their collection was unknown and the merchant was not forthcoming with that information.

**Site:** Yanqi Lake, Beijing, China (N 40° 23.67', E 116° 40.47')



**Date:** 16 August, 2013

**Species present:** Hydrilla, *Myriophyllum* sp.

**General information:** Yanqi Lake is in an amusement park area. A small electric boat was rented to explore the shore line area for plants. Because of recent rains, the water level had increased and high turbidity prevailed which limited collection success. Only a small number of floating hydrilla fragments were collected, none of which were flowering. Floating fragments were collected 10-20 m from shore. Very little surfaced aquatic vegetation was observed (<1% surface coverage). Substrate was rock/clay.



**Site:** Yanqi River, Beijing, China (N 40° 22.06', E 116° 40.19')



**Date:** 16 August, 2013

Species present: Hydrilla

**General information:** An approximately 50 m wide river with only a small amount of hydrilla growing along margin next to a bridge. Total aquatic vegetation surface cover was ~20%, with <1% hydrilla. No flowers were observed on hydrilla plants. Hydrilla samples were collected 1-3 m from shore at a water depth of 0.5-1 m. Substrate was rock/clay/silt.

**Site:** Wulie River, Chengde, Hebei, China (N 40° 57.67', E 117° 56.55')



**Date:** 17 August, 2013

**Species present:** *Hydrilla*, *Ceratophyllum* sp., *Myriophyllum spicatum*, *Nelumbo nucifera*, *Nymphaea* sp., *Potamogeton crispus*, *Typha* sp.

**General information:** The Wulie River site was approximately 100 m wide and located between Chengde and Beijing. Despite recent flooding, hydrilla was present and easily collected from the turbid water. Both male and female plants were collected but none that could be identified as monoecious. Hydrilla was collected 0.5-3 m from shore at a point where water depth ranged from 1.0-1.5 m. Aquatic vegetation provided ~20% surface coverage. Of total vegetation, hydrilla made up ~30%. Substrate was silt/clay.



**Site:** Bishu shan zhuang, Chengde, Hebei, China (N 40° 58.95', E 117° 56.32')



**Date:** 17 August, 2013

**Species present:** *Hydrilla*, *Ceratophyllum* sp., *Myriophyllum spicatum*, *Najas marina*, *Nelumbo nucifera*, *Potamogeton crispus*, *Potamogeton* sp.

**General information:** A 50 m wide ornamental pond at the Summer Palace in Chengde with high diversity of aquatic plant species. Aquatic vegetation provided 90% surface coverage, 10% of which was hydrilla. No signs of insect damage. Female hydrilla plants were collected 0.5-1.0 m from shore at a water depth of 0.5-1 m. Substrate was clay.

**Site:** Chaobaihe Bridge, Chaobaihe River, Beijing, China (N 40° 22.93', E 116° 53.45')



**Date:** 17 August, 2013

**Species present:** *Hydrilla*, *Myriophyllum spicatum*, *Polygonum* sp., *Najas marina*.

**General information:** A stop was made at Chaobaihe Bridge near Beijing. The Chaobaihe River was approximately 30 m wide at the sampling location. Aquatic vegetation covered ~20% of the water's surface, ~10% of which was hydrilla. Only female hydrilla plants were observed. Hydrilla was collected 1-2 m from shore at a point where depth was 0.5-0.7 m. Substrate was rock/clay.



**Site:** Nan hu Lake, Changchun, Jilin, China (N 43° 51.06', E 125° 17.96')



**Date:** 19 August, 2013

**Species present:** *Hydrilla*, *Nymphaea* sp., *Nymphoides* sp., *Trapa* sp., *Potamogeton* sp., *Myriophyllum* sp.

**General information:** *Hydrilla* was present at Nan hu Lake at several sites along the shoreline. The lake was approximately 500 m wide. Aquatic vegetation coverage was at ~50% while *hydrilla* made up ~10% of aquatic vegetation. Plants were examined and insect (leaf-mining) damage was recorded. Both male and female plants were collected but none were identified as monoecious. *Hydrilla* was collected 0.5-1 m from shore at a point where water depth was 0.2-0.5 m. Substrate was clay.

**Site:** Boxue Road site, Changchun, Jilin, China (N 43° 49.25', E 125° 25.63')



**Date:** 19 August, 2013

**Species present:** Hydrilla, *Ceratophyllum* sp., *Lemna* sp., *Phragmites* sp., *Trapa* sp.

**General information:** Hydrilla was more difficult to locate at the Boxue Road site than others, likely because the increased water level (due to recent flooding) submersed previously canopied plants. Plants were collected using a rake and rope from the bridge crossing. Aquatic vegetation covered approximately 10% of the water's surface, and hydrilla composed ~25% of total vegetation. Male and female flowers were observed on separate plants. Substrate type is unknown.



**Site:** Jingyue Park, Changchun, Jilin, China (N 43° 47.76', E 125° 26.77')



**Date:** 19 August, 2013

**Species present:** *Hydrilla*, *Ceratophyllum* sp., *Sagittaria* sp., *Trapa* sp., *Typha* sp.

**General information:** Lake within public park. Only male flowers observed on hydrilla. Visual examination revealed *Hydrellia* sp. damage. Aquatic vegetation was dense, covering ~80% of the water surface. Hydrilla composed ~60% of all vegetation. Hydrilla samples were taken from 0.5-1.0 m from shore and at a water depth of 1.2 m. Substrate type was clay.

**Site:** Puhe River, Huishan village, Liaoning, China (N 41° 54.91', E 123° 38.51')



**Date:** 20 August, 2013

**Species present:** Hydrilla, *Potamogeton crispus*.

**General information:** A small pond on the Puhe River, near Huishan village. Substrate near shore was rocky but there were patches of hydrilla as well as *Potamogeton crispus*. Total aquatic vegetation covered approximately 10% of the water surface, of which ~50% was hydrilla. Hydrilla was damaged by *Hydrellia* sp. as evidenced by leaf-mines. Male and female hydrilla plants were collected, but no monoecious plants were identified. Hydrilla samples were collected 0.5-1.5 m from the shore at a depth of 0.5 m.



**Site:** Nan hu Park, Shenyang (N 41° 46.03', E 123° 24.06')



**Date:** 21 August, 2013

Species present: Hydrilla

**General information:** Collections of hydrilla at Nan hu amusement park were floating stem fragments from a 15 m-wide canal. The water was turbid, presumably from recent rains. The upstream source of the fragments could not be found. Several fragments of female plants and one non-flowering fragment were collected. No other vegetation was observed. No monoecious plants were identified.

**Site:** Dong hu Lake, Shenyang, Liaoning, China (N 41° 49.64', E 123° 35.66')



**Date:** 21 August, 2013

**Species present:** *Hydrilla*, *Ceratophyllum* sp., *Nelumbo nucifera*, *Sagittaria* sp., *Typha* sp.

**General information:** Both male and female plants were collected from shallow margins of the lake, but no monoecious plants were identified. Stem damage was observed, but no associated herbivores were identified. *Hydrellia* sp -mined leaves were commonly observed. Approximately 90% of the water surface was covered in aquatic vegetation; ~10% of aquatic vegetation was hydrilla. Hydrilla plants were sampled 0.5-1 m from shore and at points where the depth was 0.3 m. Substrate was silt/clay.



**Site:** Tang River, Qijiatun village, Liaoning, China (N 41° 13.80', E 123° 22.34')



**Date:** 22 August, 2013

**Species present:** *Hydrilla*, *Ceratophyllum* sp., *Lemna* sp., *Potamogeton crispus*, *Potamogeton* sp.

**General information:** Plants were collected from a bridge crossing above the Tang River in a small village west of Benxi and south of Shenyang. A throw rake was used to sample plants in the fast-flowing water below. Both male and female plants were collected, but no monoecious plants were identified. Approximately 10% of the water's surface was covered by aquatic vegetation; hydrilla made up ~10% of the aquatic plants at this site. Hydrilla plants were collected 5.0-15.0 m from the bridge. Substrate was rocky/clay.

**Site:** Highway G203, Roadside irrigation ditch, 14 km S of Songyuen, Jilin, China (N 45° 02.70', E 124° 36.51')



**Date:** 24 August, 2013

**Species present:** Hydrilla, *Ceratophyllum* sp., *Lemna* sp., *Potamogeton* sp., *Typha* sp.

**General information:** This collection site was the result of observing canopied aquatic plants in a roadside irrigation channel while driving to Tongliao in Inner Mongolia. Small patches of flowering hydrilla mixed with extensive *Ceratophyllum* sp. were examined for herbivory. Male and female hydrilla plants were collected but no monoecious plants were identified. Total surface cover from vegetation was ~60%. Hydrilla composed ~5% of aquatic vegetation at this site. Hydrilla plants were collected by a throw-rake at a distance of 3-5 m from shore. Water depth was not recorded. Substrate was clay.



**Site:** Chagan hu Lake, Jilin, China (N 45° 12.22', E 124° 25.63')



**Date:** 25 August, 2013

**Species present:** *Hydrilla*, *Ceratophyllum* sp., *Nelumbo nucifera*, *Nymphaea* sp., *Phragmites* sp.

**General information:** Chagan hu Lake is a tourist destination. No hydrilla could be found along the shoreline of the main lake, but hydrilla was collected from a small adjacent pond in a public area. Lake personnel were observed hand-harvesting hydrilla from a boat, possibly to create boat lanes. Hydrilla growth, along with *Nelumbo nucifera*, was very dense. Aquatic vegetation covered approximately 70% of the water's surface; hydrilla made up ~20% of total vegetation. Male and female plants were collected but no monoecious plants were identified. Fish had heavily defoliated hydrilla 20 cm below the water surface. Plants were collected 0.5-1.0 m from a pier, where the water was 1.0 m deep. Substrate was clay.

**Site:** Harbin Fun Park, Harbin, Heilongjiang, China (N 45° 46.82', E 126° 39.79')



**Date:** 28 August, 2013

**Species present:** *Hydrilla*, *Myriophyllum* sp., *Typha* sp.

**General information:** This site is a pond, approximately 100 m wide, within a fun park in the city of Harbin. Small patches of hydrilla and *Myriophyllum* were examined. Male and female hydrilla plants were collected but no monoecious plants were identified. Small ephydrid flies were aspirated from the water surface and along the wall at the edge of the pond. Hydrilla stems were thinner (smaller stem diameter) with fewer leaves than found elsewhere. Total aquatic vegetation surface coverage was low, at ~5%. Hydrilla made up a small portion of total vegetation (<5%). Substrate was clay.



**Site:** Dingjiashang Village, Hunan Province (N 29°36.92', E 111°28.98')



**Date:** 13 September, 2013

**Species present:** Hydrilla (monoecious), *Potamogeton* sp.

**General information:** This site is an irrigation canal. Hydrilla formed large mats at the water surface with *Potamogeton* sp. growing at the mats' edges. Hydrilla flowers were present at the water surface, and monoecious plants were collected. It could not be determined if all hydrilla plants growing at this site were the monoecious biotype.



**Site:** Wuxi Village, Hunan Province (N 29°04.45', E 110°21.52')



**Date:** 14 September, 2013

**Species present:** Hydrilla

**General information:** The hydrilla was submerged (not canopied) and covered with sediment at a river bend. No flowers were observed on hydrilla plants but some leaves were damaged by moth larvae.

**Site:** Pengjiazhaishang Village, Hunan Province (N 28°55.52', E 110°00.89')



**Date:** 14 September, 2013

**Species present:** Hydrilla, *Marsilea quadrifolia*, *Polygonum* sp., *Potamogeton* spp.

**General information:** A shallow river site with abundant hydrilla plants and a few *Potamogeton* spp. and *Marsilea quadrifolia* plants mixed together. The plants were sediment-covered and emerged from the water surface. Few flowers were found on hydrilla; most observed flowers were female. Only one stem of monoecious hydrilla was found.

**Site:** Gaoping Town, Henan Province (N 28°50.78', E 109°58.63')

**(No photograph available)**

**Date:** 14 September, 2013

**Species present:** Hydrilla

**General information:** This site is a bridge crossing of a river. Hydrilla was fully submerged (i.e., not canopied) and sediment-covered. No flowers were observed on hydrilla plants.

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**Site:** Tuxipu Village, Hunan Province (N 26°46.55', E 109°42.25')



**Date:** 15 September, 2013

**Species present:** Hydrilla

**General information:** A river site with robust, green hydrilla. No flowers were observed on hydrilla plants. Smaller, brown hydrilla plants were also present; these had male, female or both flowers present. Monoecious plants were collected.



**Site:** Shiwaitaoyuan Park, Guangxi Province (N 24°52.02', E 110°23.05')



**Date:** 17 September, 2013

**Species present:** Hydrilla, *Eichhornia crassipes*, *Polygonum* sp., *Vallisneria natans*

**General information:** This site is a river behind the Shiwaitaoyuan Park. Hydrilla, *Vallisneria natans* and *Polygonum* sp. were growing in mixed beds. Some *Eichhornia crassipes* plants floated near the shore. All observed hydrilla flowers were male.

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14. ABSTRACT  This report summarizes the result of 2013 surveys in the People's Republic of China of the invasive aquatic weed <i>Hydrilla verticillata</i> for potential insect biological control agents. Plants were examined at 34 sites throughout eastern China, resulting in the collection of leaf-mining <i>Hydrellia</i> spp. (Diptera: Ephydriidae), <i>Parapoynx diminutalis</i> (Lepidoptera: Crambidae), various Chironomidae (Diptera), <i>Macrolea japonica</i> (Coleoptera: Chrysomelidae), and other unidentified insect herbivores. Additionally, effort was made to identify plants as monoecious or dioecious, since both biotypes are present in the US. However, at the majority of sites only dioecious plants were apparent. Previous surveys in China were limited in scope and never included biotype identification; consequently, the present work represents an initial attempt to locate biotype-matched hydrilla biological control agents for use in the US. An Appendix with site locations and descriptions is included.					
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