Potential biological control agents matched for invasive hydrilla biotypes in the USA

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CSIRO HEALTH AND BIOSECURITY

Outline of presentation

- What is USDA ARS Australian Biological Control Laboratory
- Brief overview of what is biological control
- Hydrilla the problem, current status of biological control
- Hydrilla surveys in the Peoples Republic of China (PRC) and the Republic of Korea (ROK)
- Potential biological control agents
- Planned and potential future research

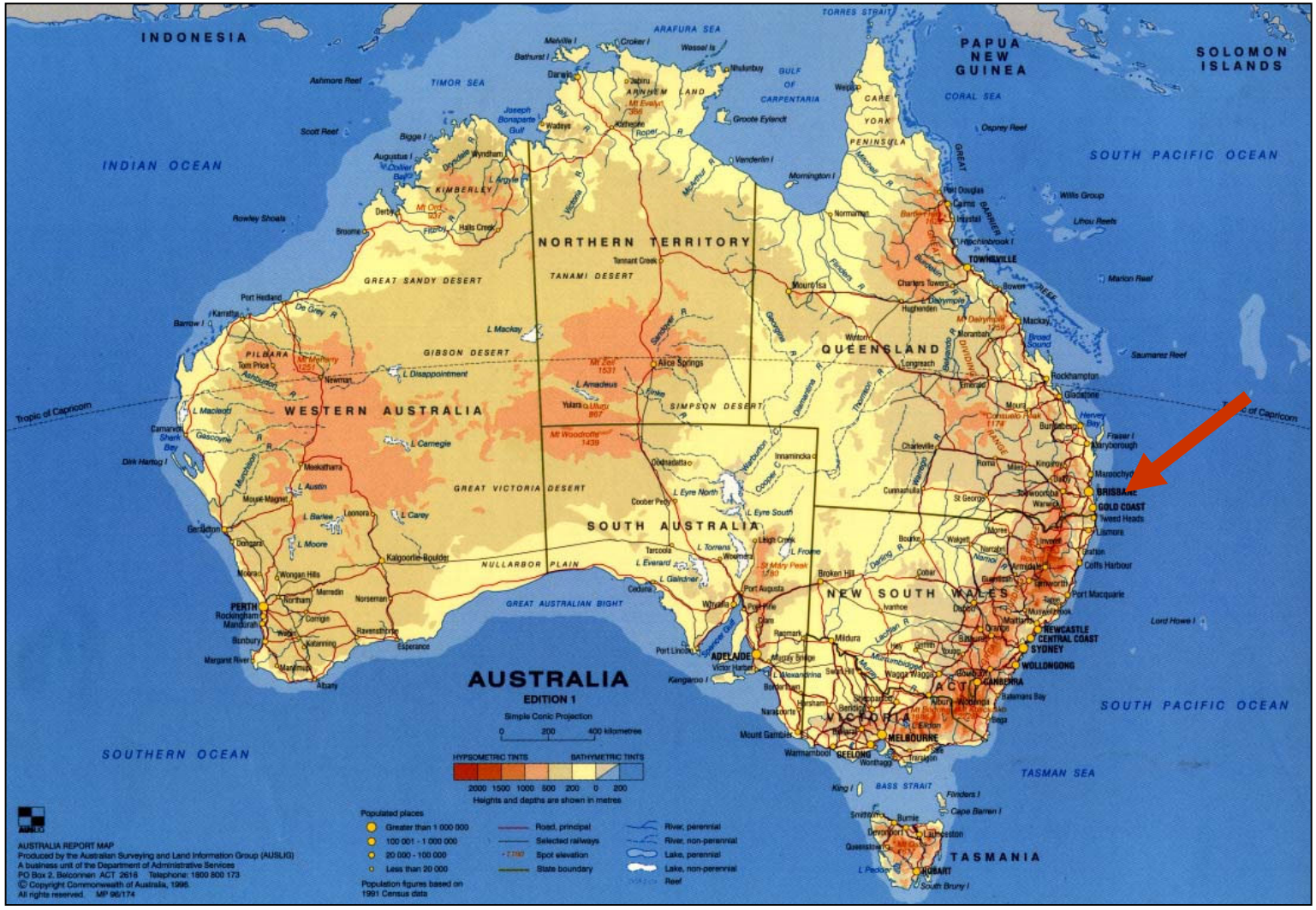
USDA ARS
Overseas Biological Control Laboratories

USDA ARS Overseas Laboratories

- Australian Biological Control Laboratory (ABCL)
- European Biological Control Laboratory (EBCL)
- Sino America Biological Control Laboratory (Sino ABCL)
- FuEDEI – ex South American Biological Control Laboratory (SABCL)

Mission: To conduct research projects to search for, identify, evaluate and prioritize potential biological control agents for use against US invasive species

Where is ABCL?



Ecosciences Precinct, Brisbane

CSIRO Structure

Research Business units

- Agriculture & Food
- **Health & Biosecurity**
- Land & Water
- Oceans & Atmosphere
- Mineral resources
- Energy
- Manufacturing
- Data 61

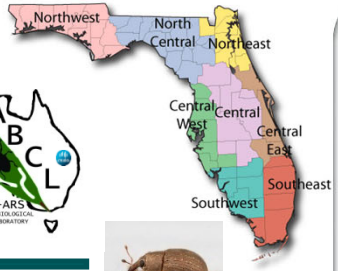
National Facilities & Collections

- Australian Animal Health Lab
- **National Biological Collections**
- Astronomy & Space Science
- Marine Research Boat
- Information Management & Technology

Global centres

- France
- **USA**
- Chile
- Indonesia
- China
- Singapore

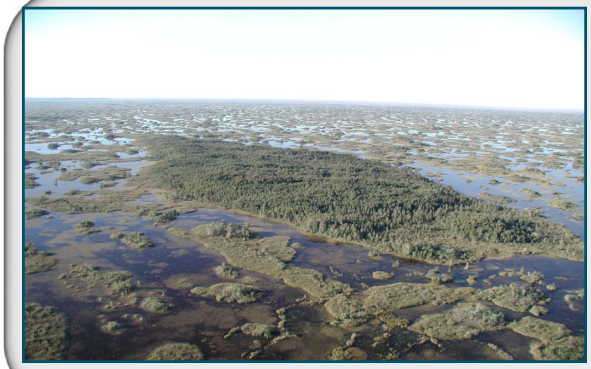
Overview of ABCL



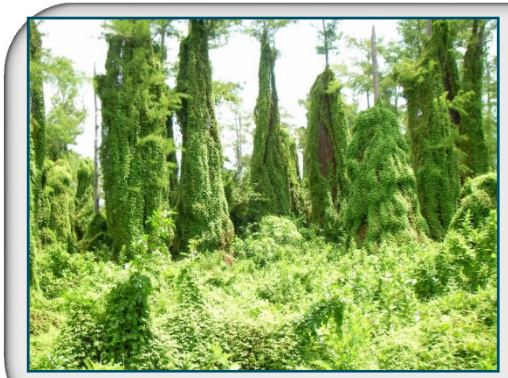
Korea

China with ERDC

Philippines



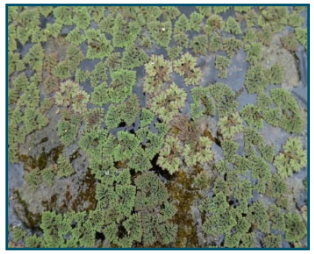
Melaleuca quinquenervia
melaleuca



Lygodium microphyllum,
old world climbing fern

Acacia auriculiformis
ear leaf acacia

Rotala rotundifolia
roundleaf toothcup



Azolla pinnata
red azolla

Hydrilla verticillata
hydrilla

Nymphoides cristata
crested floating heart

Nymphoides peltata
yellow floating heart

ABCL Regional Exploration

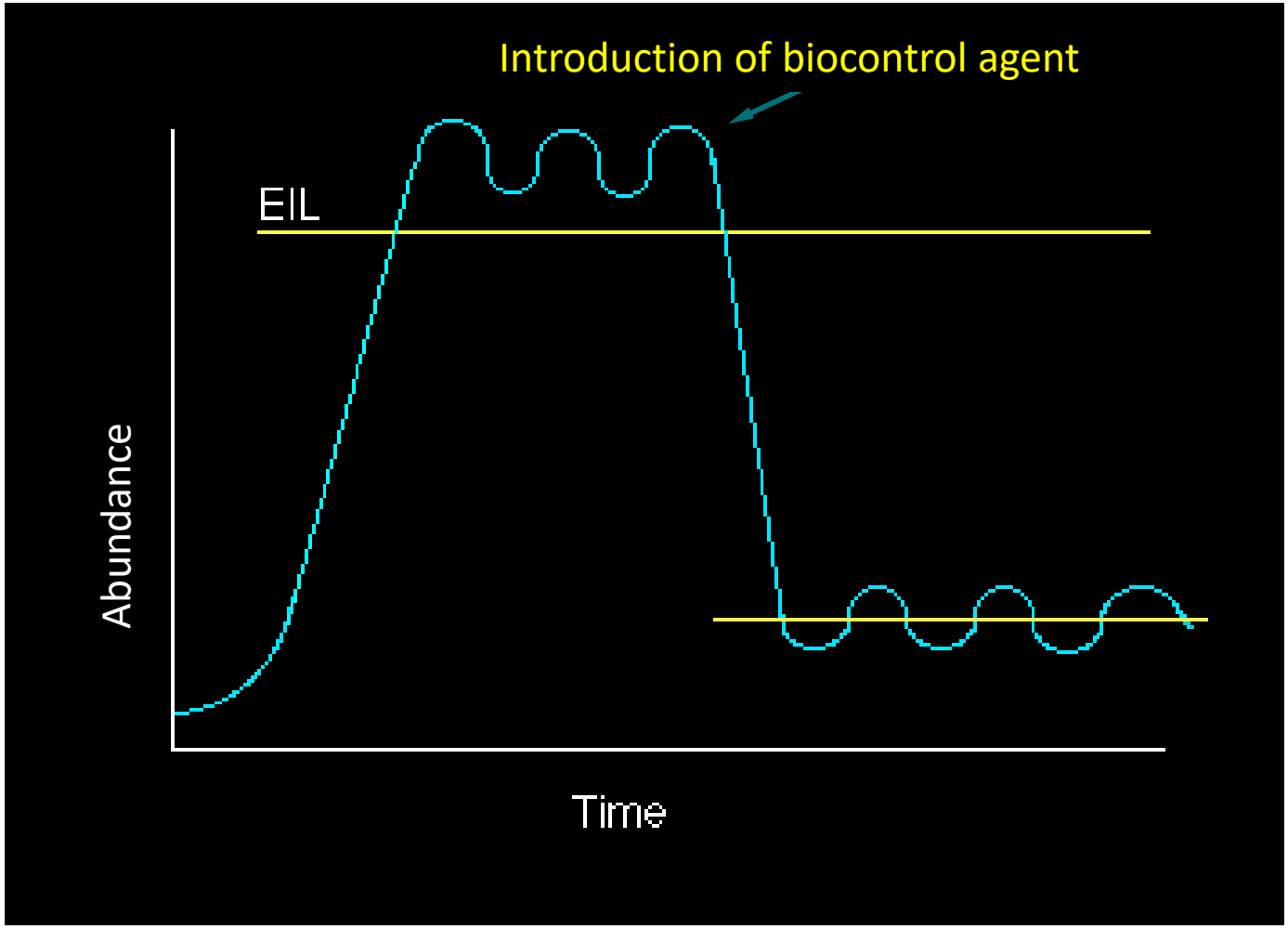
What is Classical Biological Control?

Definition:

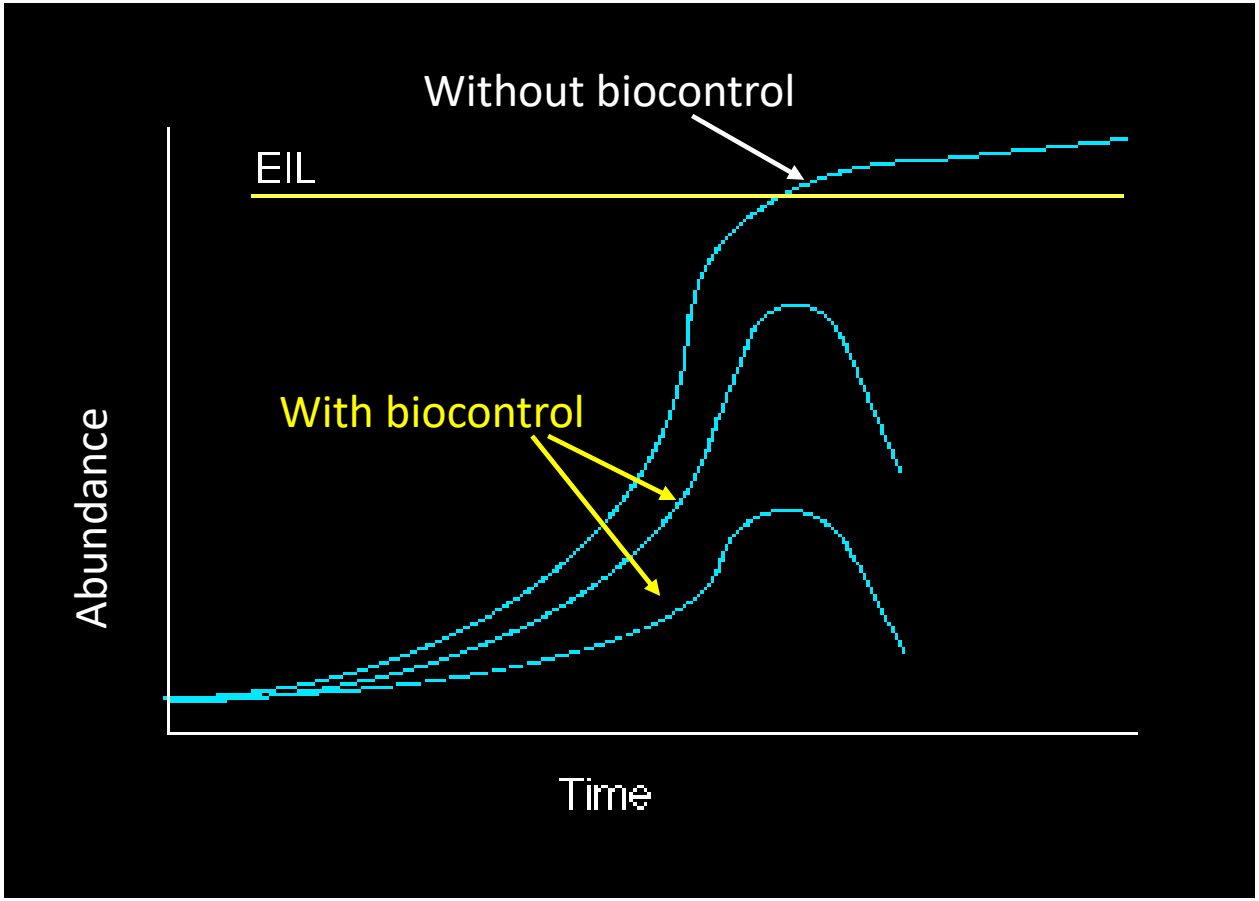
The intentional introduction of an exotic biological control agent for permanent establishment and long-term control of pest/weed populations

- Requires initial importation and release of natural enemies
- There are two types of management
  - Reduction and prevention

Reduction



Prevention

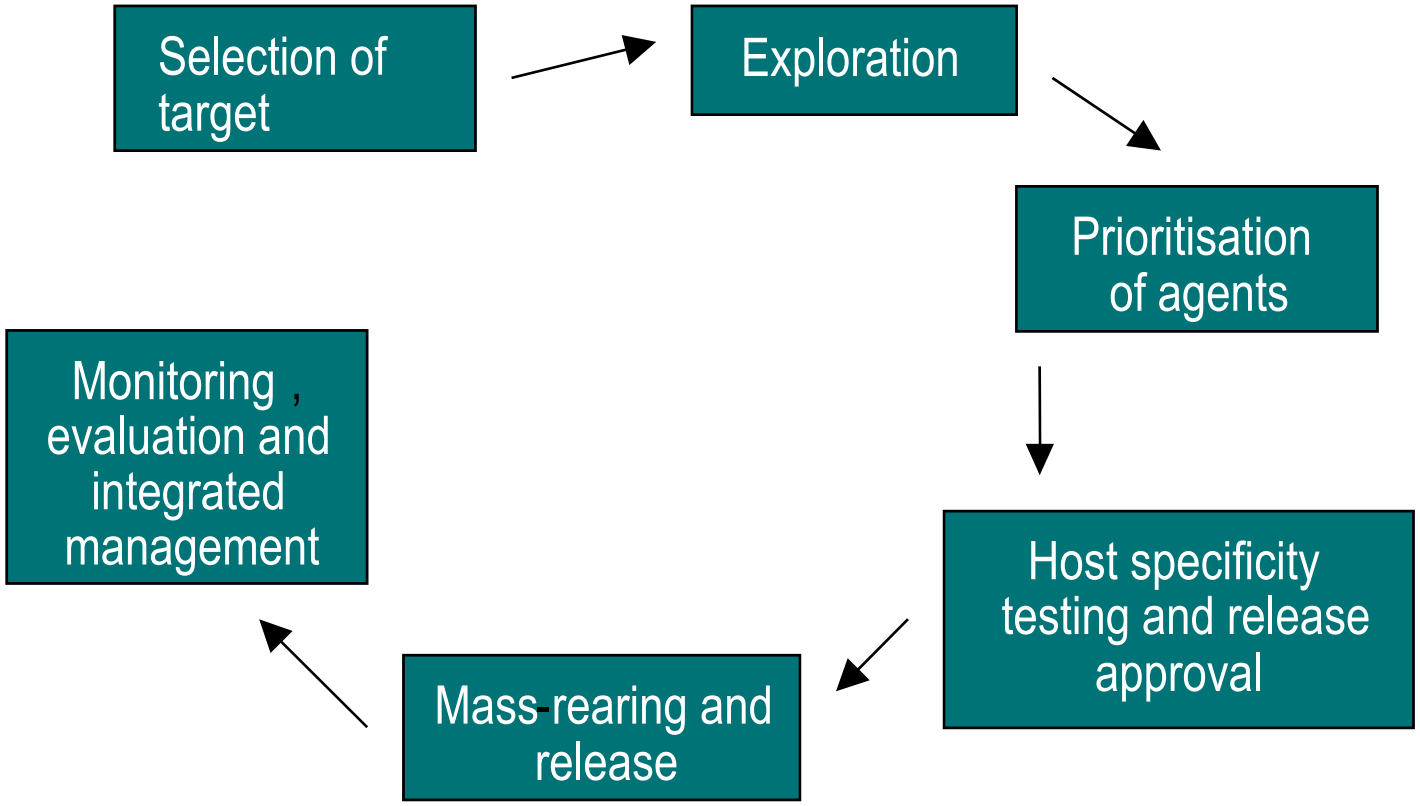


Enemy Release Hypothesis

- Exotic species are limited by its natural enemies in its native range
- In the process of being moved from native to the introduced range, natural enemies are left behind
- This leads to improved competitiveness in the invasive range which then leads to invasiveness (weeds and pests)
- When the escape from natural enemies is the cause of invasiveness, Classical Biological Control (CBC) can potentially be a powerful management solution

Steps in Classical Biological Control

Photos: Biosecurity Queensland

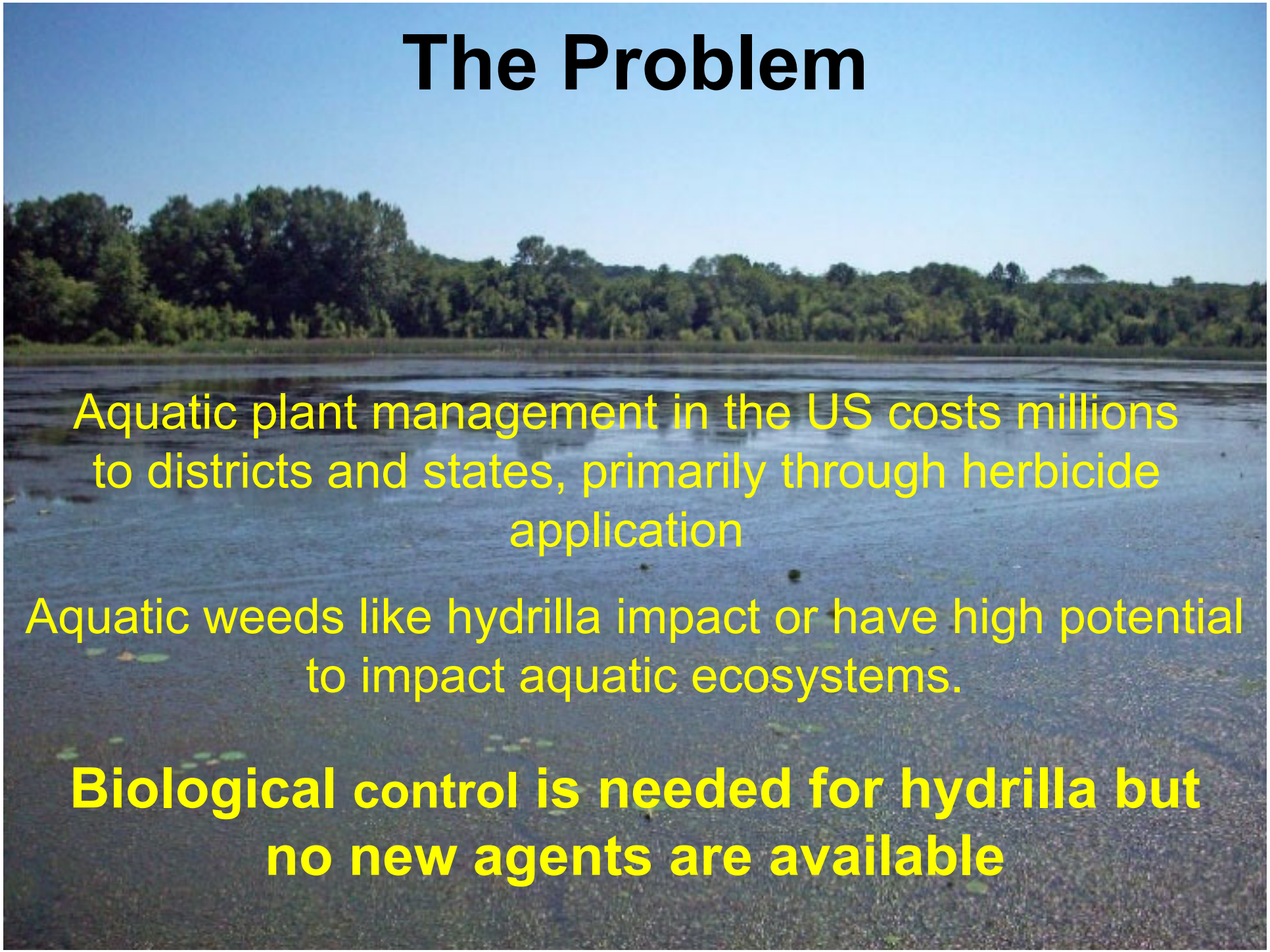


Steps in Classical Biological Control

Native Range Studies

- Understanding pest/weed ecology
- Understanding natural enemy complex
- Assessing role of natural enemies, relative to other factors identified in demography studies
- Preliminary evaluation of the host range and impact of natural enemies both in the Lab, field, greenhouse, open-field)
- Understanding life-history requirements of specialist natural enemies Shipping of natural enemies (agents) to exotic range

The Problem



Aquatic plant management in the US costs millions to districts and states, primarily through herbicide application

Aquatic weeds like hydrilla impact or have high potential to impact aquatic ecosystems.

Biological control is needed for hydrilla but no new agents are available

Hydrilla in the USA and biocontrol

- In US since 1950s, expanded from Florida to Delaware and westward to Texas and California
- Three genotypes (and two biotypes, dioecious and monoecious) recognized in US
- Four insect agents released for biocontrol, only the leaf-mining flies have permanently established **and only partial control achieved and these have an even smaller impact on monoecious hydrilla**

Rarely problematic in the native range

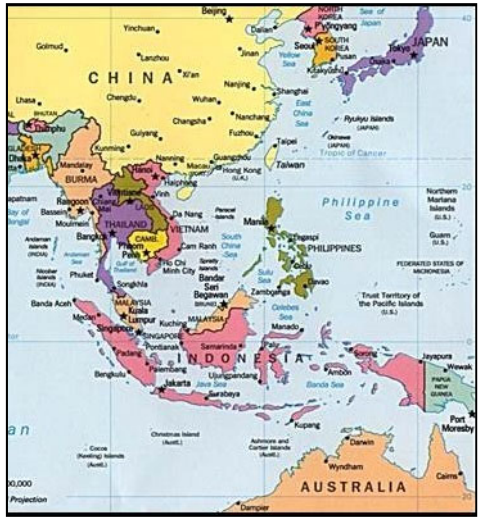
Negative impacts on irrigation, navigation, and wildlife

Why we are surveying in Asia?

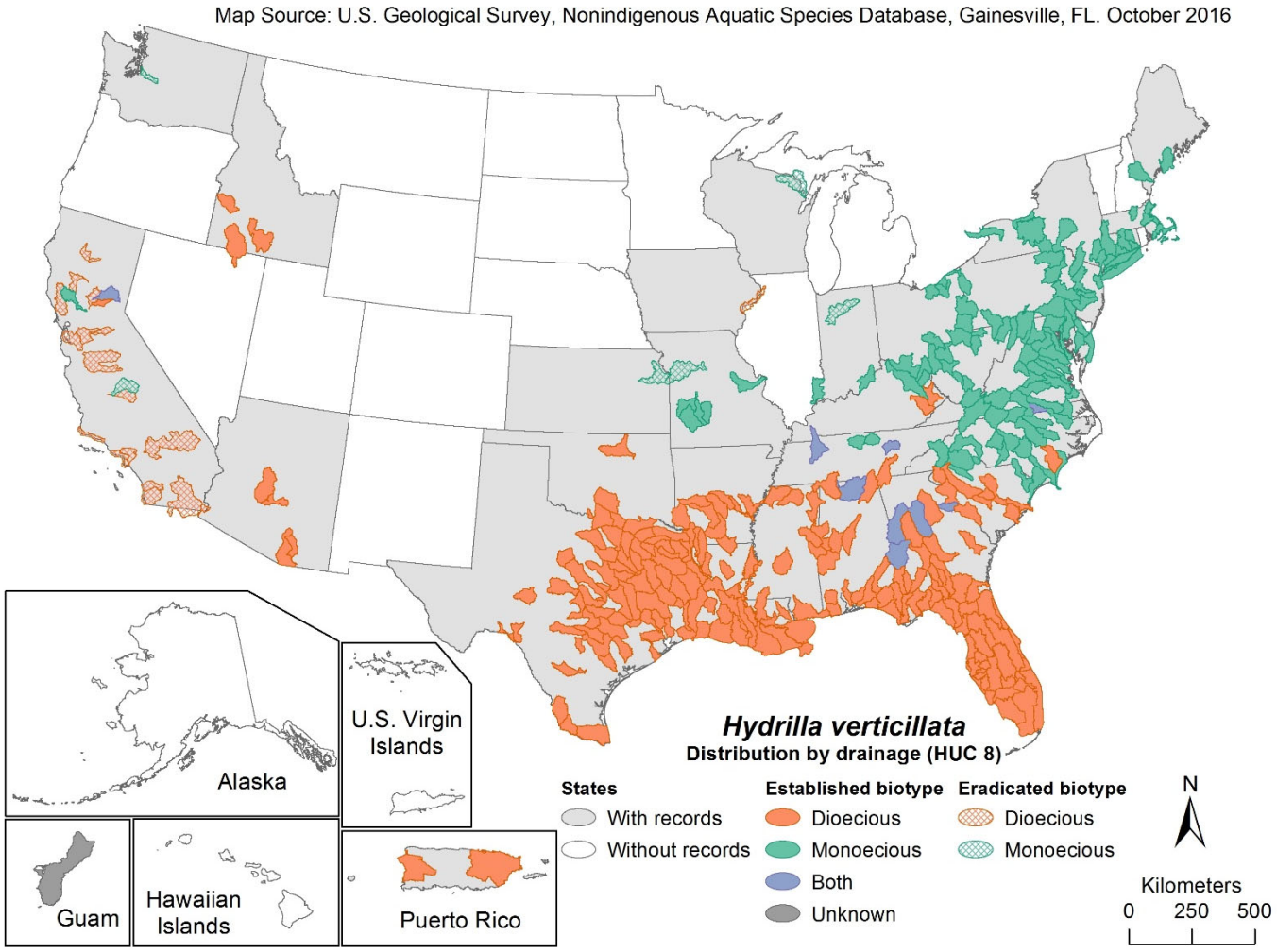
- Substantial surveys were conducted on the Indian sub-continent, Asia and Australia in the 60's, 70's and 80's and agents were selected and released
- Surveys conducted for biocontrol agents in Africa found only non-specific Lepidoptera and tip midges (Polypodium); despite the lack of diversity, some botanists believed that Africa could be the center of origin for Hydrilla
- However, an alternative hypothesis suggested that Asia was the area of origin for hydrilla.
- Genetic characterization of hydrilla indicated that the greatest diversity of hydrilla occurs in China
- Only limited surveys had been conducted in China and Korea in the late 80's
- New surveys were initiated by USDA ARS in Asia in 1996 with an objective to complete hydrilla exploration

Initial exploration efforts in Asia

- Surveys for biological control agents included almost 400 collections of hydrilla in Australia, China, Indonesia, Malaysia, Singapore, Thailand and Vietnam between 1996 and 2013 (Purcell et al 2019)
- Many non-specific herbivores were collected and tested though selected foliage feeding moths, weevils, flies and mites could warrant further investigation



Monoecious hydrilla in the US



Collaboration with the US Army Engineer Research and Development Center

US Army Corps of Engineers®
Engineer Research and Development Center

Hydrilla verticillata
hydrilla

Nymphoides cristata
crested floating heart

Nymphoides peltata
yellow floating heart

Why we are surveying in PRC and ROK?

- The monoecious biotype of hydrilla was believed to have originated from ROK
- Recent genetic characterisation of hydrilla samples collected from PRC and ROK by USDA ARS ABCL/USACE ERDC have located sites in both countries which contain hydrilla matching the **US monoecious (known as mono21-21) and dioecious biotypes (dio2-25)**
- Genetic characterization has indicated that the **greatest genetic diversity of hydrilla** occurs in PRC which could support a greater array of insect fauna and potential biological control agents

Collaborators

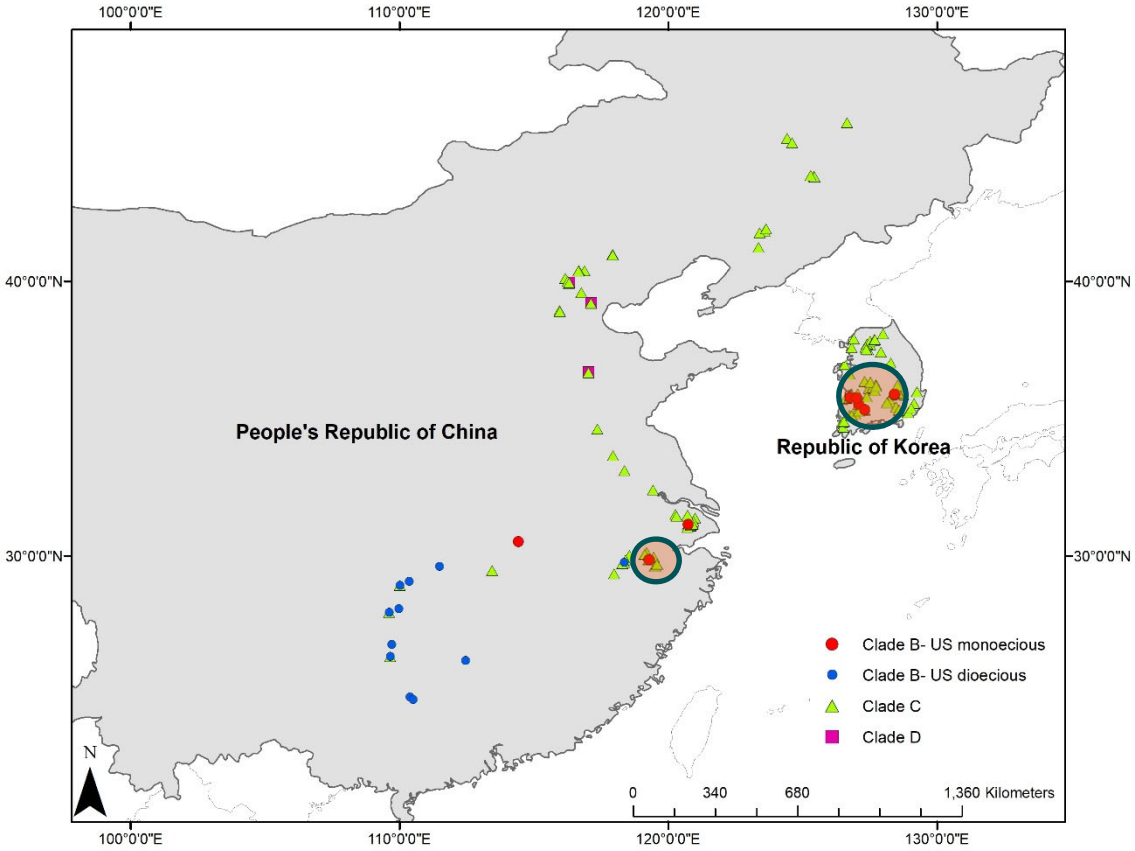
Henan University

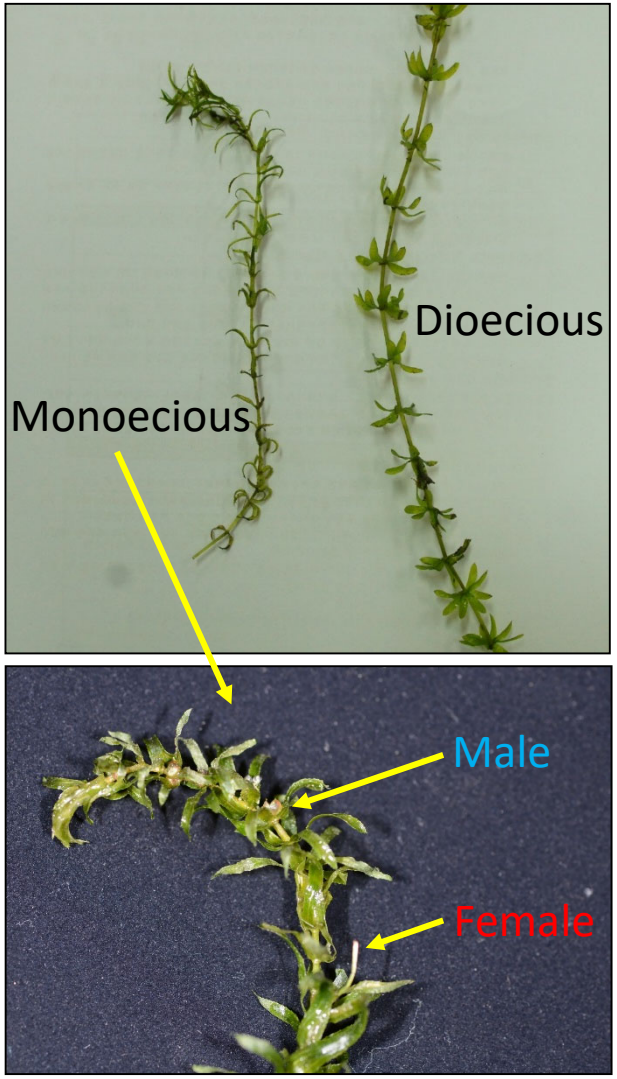
Hankyong University

Chinese Academy of Science

Hydrilla surveys in PRC/ROK

We collected hydrilla (n = 592 samples) from a total of 66 sites in PRC (n = 320 samples) and 63 sites in ROK (n = 276 samples) during 2013-2015 (Williams et al. 2018)





Genetic characterisation of hydrilla

Clade B
(5 haplotypes)

US Monoecious (mono21-21) PRC/ROK

US Dioecious (dio2-25) PRC

3 Haplotypes PRC

Monoecious (n=10, 3 haplotypes)
Dioecious (n=18, 4 haplotypes)
2 shared haplotypes

Clade C
(19 haplotypes)

2 Haplotypes ROK

3 Haplotypes PRC/ROK

14 Haplotypes PRC

Dioecious (n=63, 10 haplotypes)

Genetic characterisation of hydrilla

Other Interesting Observations

- Both monoecious and dioecious forms were found together at the same site
- Monoecious and dioecious forms shared haplotypes, this included dio21-21
- Plants from both Clade B and Clade C were found together at some sites. Are they sexually incompatible or separated ecologically (e.g. flowering at different times)?

Subsequent PRC and ROK surveys

- Monthly monitoring of field sites during the hydrilla growing season by collaborators **monitoring recording insect herbivores and their damage** in ROK and PRC as well as plant phenology parameters

- Surveyed **new areas** in Korea and collected hydrilla samples for genetic analysis

- Collected prioritised biocontrol agents of monoecious hydrilla and hand carried them to quarantine in Australia

Biocontrol of monoecious hydrilla

Existing agents **aren't effective against monoecious hydrilla**

Ephydridae: *Hydrellia* spp.

Hydrilla dieback in winter

- The leaf-mining *Hydrellia* flies are the only biological control agents established on hydrilla in the US
- These flies fail to overwinter on monecious hydrilla in cooler regions of the US as hydrilla dies back in winter **preventing significant populations to establish that could cause effective damage**

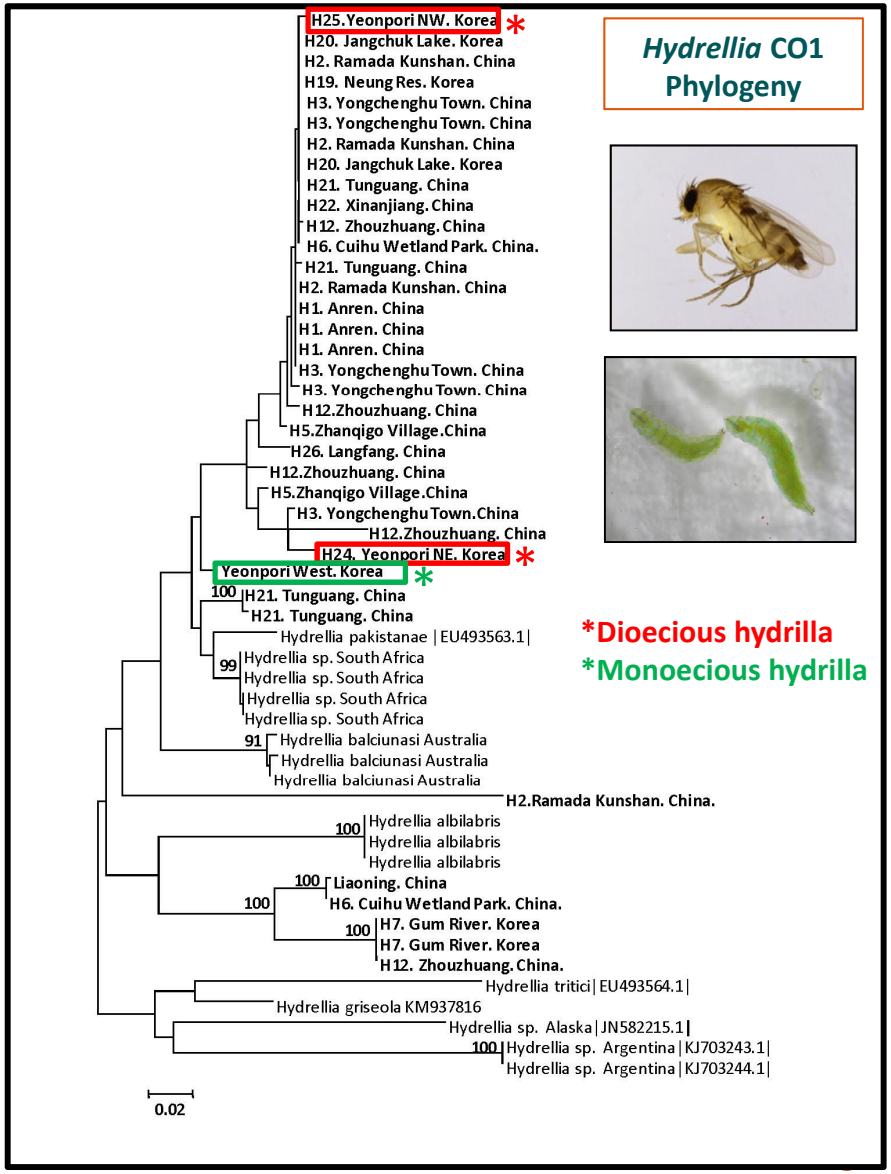
Hydrilla herbivores - *Hydrellia*

Yeongpuri NW Site

Spring regrowth of monoecious hydrilla on substrate

Berlese extraction of herbivores

Hydrellia larvae extracted



Hydrilla herbivores: leaf mining flies

Hydrellia sp.

ABCL quarantine, Brisbane, Australia

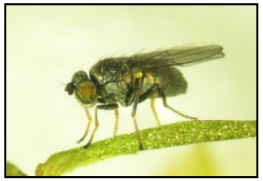
Hydrellia cultures

- *A Hydrellia* sp. genotype adapted to US monoecious form (mono21-21) has been collected in South Korea and **imported into quarantine in Brisbane, Australia**
- Overwinters on hydrilla, should be **more effective in cooler regions than *Hydrellia pakistanae***
- US monoecious form tubers have been imported from the US and plants grown in ABCL quarantine in Australia for Hydrellia **performance comparisons** between US and Australian hydrilla genotypes
- The fly has been **colonised** (20 generations) and preliminary biology and host range testing is almost completed

Hydrellia sp. in ABCL quarantine

- Egg to adult development is 19 days, adults live for 12 days
- Fast development, multiple generations per year
- And will **re-establish populations quickly after winter**
- Mobile, fast flying, readily disperses and colonises new sites

Adult emergence & mating



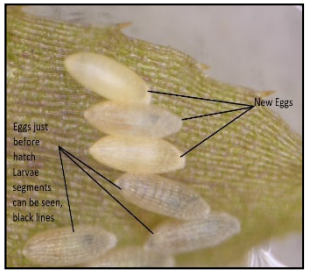
Pupa

Life cycle

Egg

Larval development

Egg hatch



Performance on host plant genotypes

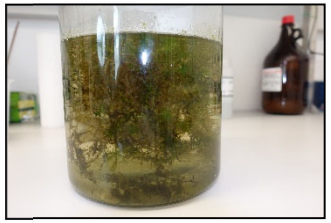
- *Hydrellia* sourced from hydrilla mono21-21 in Korea **could perform better on US monoecious hydrilla than *H. pakistanae***
- Performance of *Hydrellia purcelli* on monoecious hydrilla introduced into South Africa from Indonesia/Malaysia was **significantly better than *H. pakistanae* released in the US** from Indian subcontinent origins
- Hydrilla mono21-21 tubers were imported from the US and plants grown in ABCL quarantine for Korean *Hydrellia sp* performance comparisons between US and and Australian monoecious hydrilla genotypes

2019 – quarterly inspections by AQIS pathologists

Host range testing – mono 21-21 *Hydrellia* sp.

Plant species	No. tests	Average emergence/test	No. tests to be completed
Hydrilla verticillata	37	125	-
Egeria densa	6	0	0
Vallisneria gracilis	3	0	0
Elodea canadensis	13	122	0*
Vallisneria americana	4	0	0
Najas tenuifolia	5	0	0
Limnobium laevigatum	4	In progress	4
Ottelia ovalifolia	3	In progress	4
Hydrocharis dubia	0	-	4
Potamogeton crispus	0	46	4
Myriophyllum aquaticum	3	0	0

Hydrilla



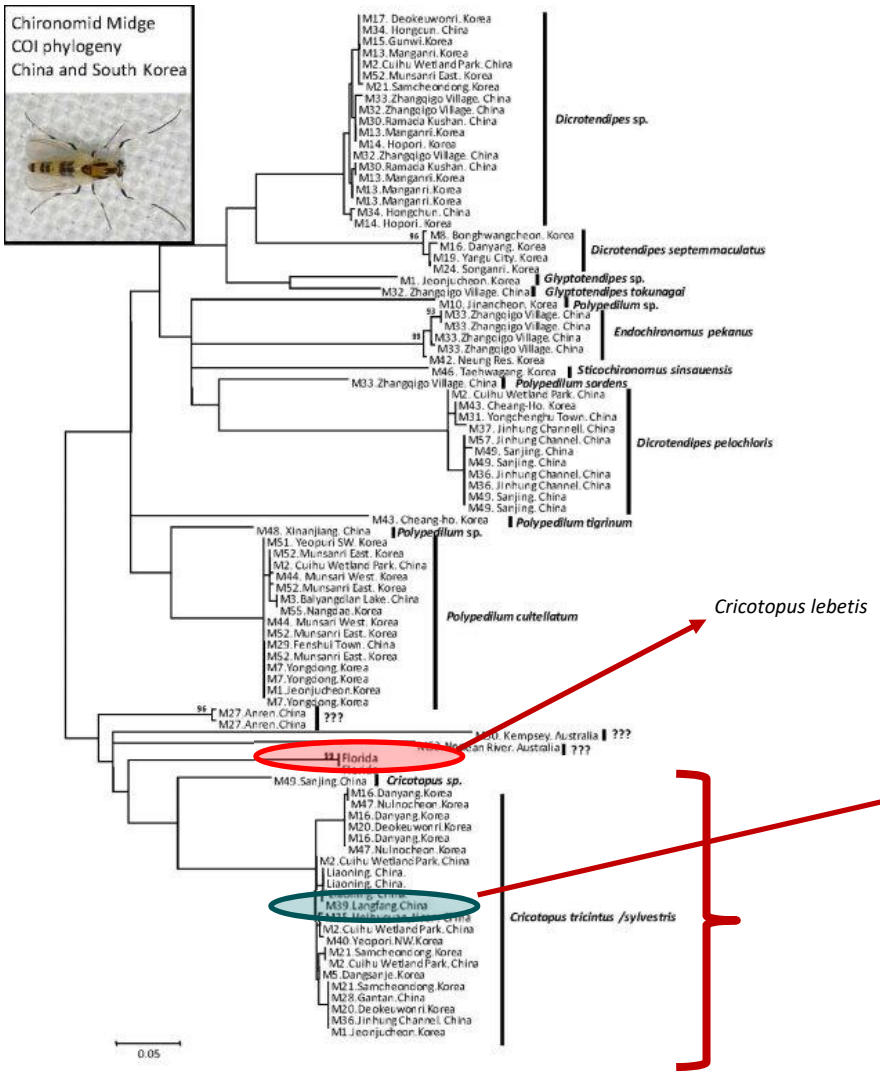
Elodea

*** Multi generational tests are underway**

All plant species test are Hydrocharitaceae except, *Potamogeton crispus* (Potamogetonaceae) and *Myriophyllum aquaticum* (Haloragaceae)

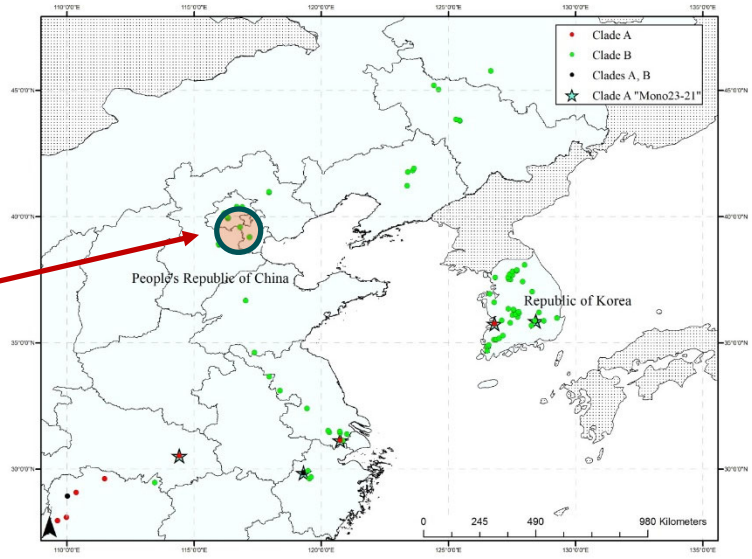
Hydrilla herbivores: midges

- Chironomidae **defoliation of hydrilla** in China

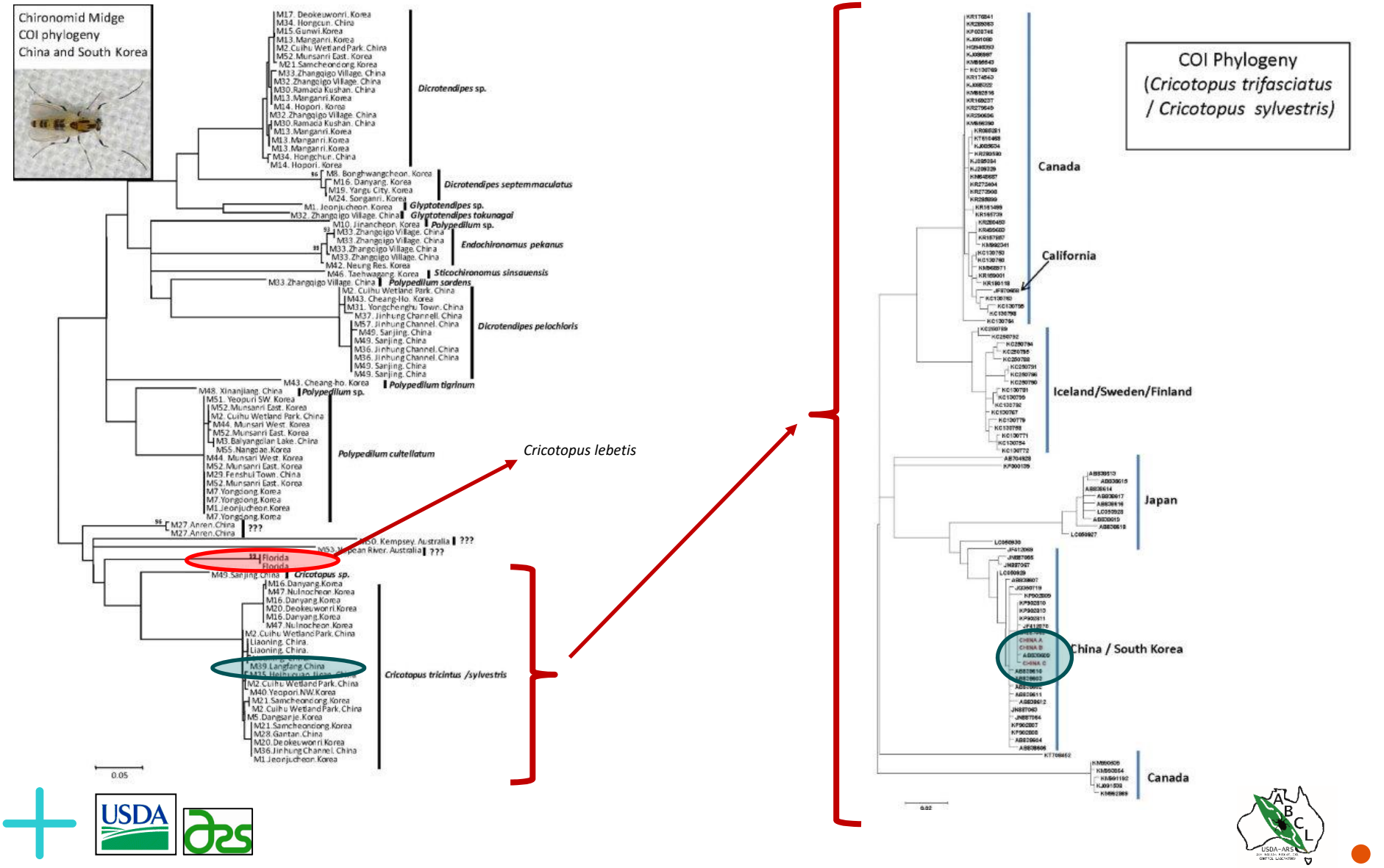


Cricotopus larvae extracted from Langfang hydrilla material

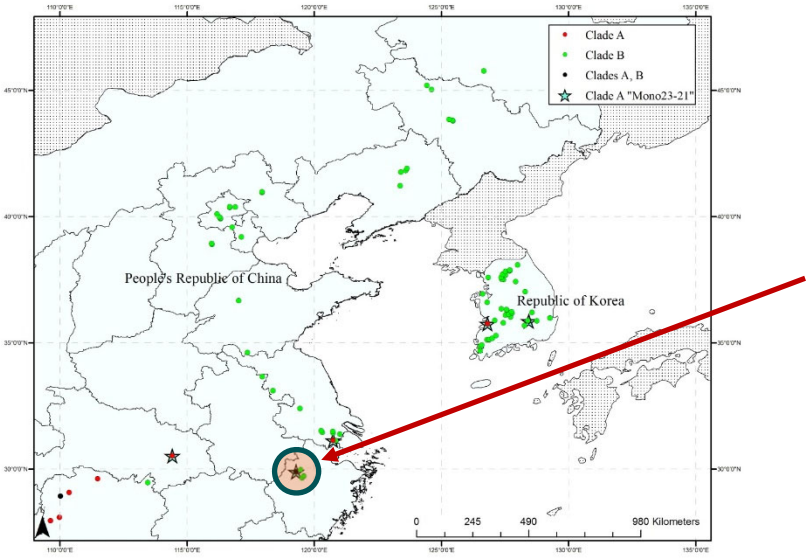
Cricotopus lebetis



Insect herbivores - midges



Insect herbivores: aquatic Crambidae



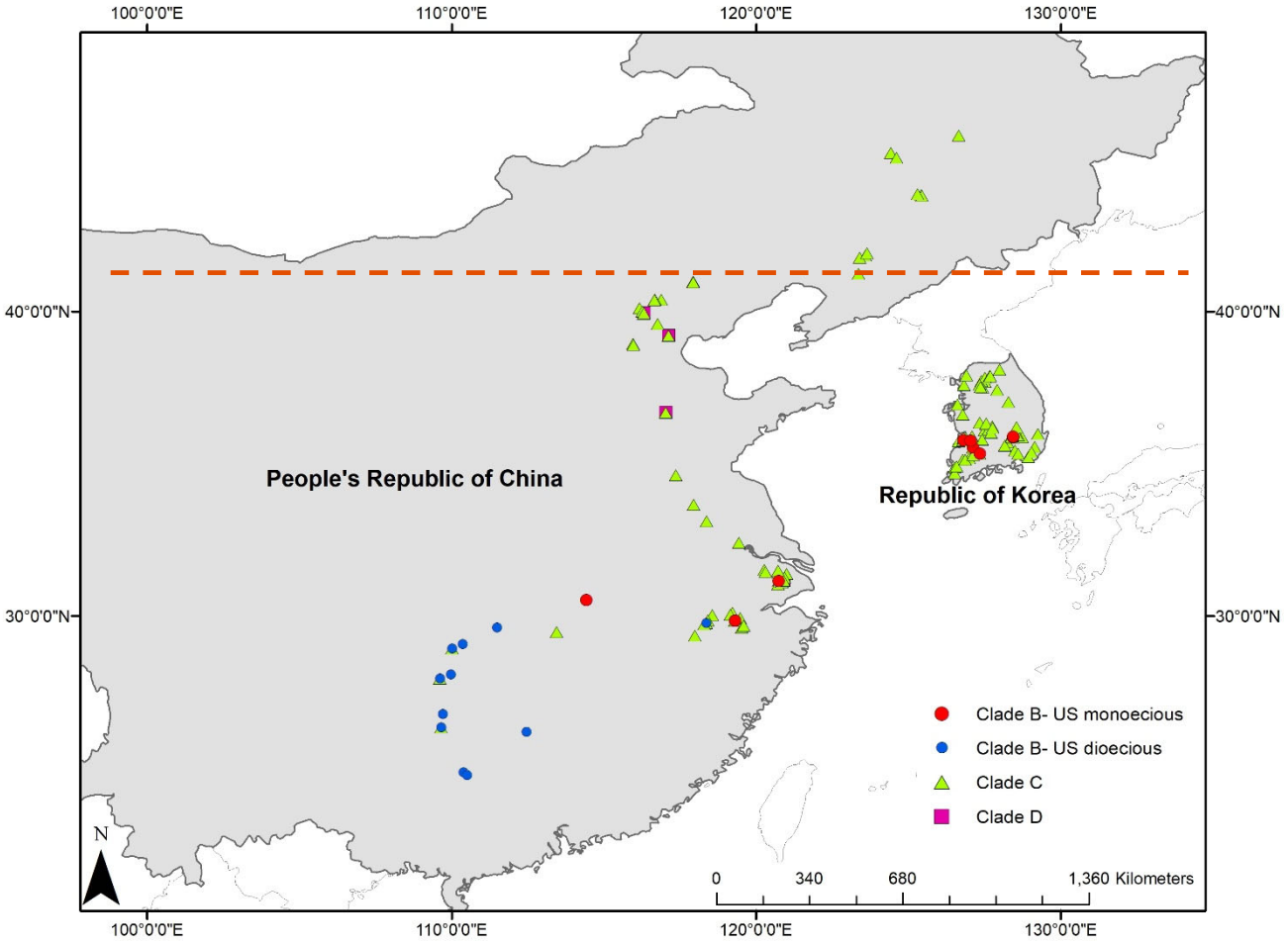
Mono21-21 at Houhe, PRC

Parapoynx sp. reared from larvae defoliating monoecious hydrilla at Houhe, PRC

New hydrilla introduction in the US

- A new (3rd) introduction of hydrilla into the US in the Connecticut River has been discovered
- The Connecticut River hydrilla plants are distinctly different from all known North American plants and is a novel introduction, likely from Eurasia (Tippery 2020)
- Plant samples are being tested (Dr Dean Williams) to see where it falls in the hydrilla phylogeny though it's in Clade C not Clade B that contains the previous monoecious and dioecious introductions
- The genotype identified (17-8) occurs in PRC and has been surveyed for natural enemies in the native range as have the closely related relatives within Clade C hydrilla occurring in China and the Republic of Korea
- Adapted herbivores could be available for biological control
- The performance of the existing agents (e.g. *Hydrellia pakistanae*) should be evaluated on this new introduction!
- **The natural enemies of Clade C hydrilla from cooler climates in China and Korea should be evaluated.**

New hydrilla introduction in the US



Clade C herbivores

Order	Family	Species	No. PRC Sites	No. ROK sites
Coleoptera	Curculionidae	*Bagous* sp.	1	1
Diptera	Ephydridae	*Hydrellia* Clade A	8	4
		Hydrellia Clade B	1	-
		Hydrellia Clade C	1	-
		Hydrellia Clade D	1	2
	Chironomidae	*Cricotopus* sp.	1	-
		Cricotopus sylvestris	3	1
		Dicrotendipes pelochloris	2	1
		Dicrotendipes septemmaculatus	-	1
		Dicrotendipes sp.	2	3
		Endochironomous pekanus	1	1
		Glyptotendipes sp.	-	1
		Glyptotendipes tokunagai	1	-
		Polypedilum cultellatum	2	2
		Polypedilum sordens	1	-
		Polypedilum sp. 1	1	-
		Polypedilum sp. 2	-	1
		Polypedilum tigrinum	-	1
		Sticochironomous sinsauensis	-	1
Lepidoptera	Crambidae	*Parapoynx diminutalis*	7	9
		Crambidae sp.	-	1
Acarina	?	?	-	1

Summary

- We have conducted extensive surveys in Asia, particularly PRC and ROK
- We have identified sites that contain all three of the US introduced genotypes of hydrilla and identified herbivores for each
- Preliminary testing is underway and planned for the cold tolerant herbivorous species attacking monecious hydrilla (*Hydrellia* flies)
- Further research is required to look at the genetic diversity of poorly understood insect herbivore guilds (e.g. aquatic crambid moths)
- Field surveys could be redirected to Clade C hydrilla in PRC/ROK to expand on our knowledge of insect herbivores

Appreciation

- U.S. Army Corps of Engineers, ERDC
- Chinese Academy of Sciences, Wuhan, China
- Henan University, Henan, China
- Korea University, Seoul, South Korea
- Hankyong University, Anseong, South Korea