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# Progress Report on Field Surveys to Identify Biocontrol Agents of *Hydrilla verticillata* in China during 2010

***by Jianqing Ding, Jialiang Zhang, and Wei Huang***

**Introduction:** *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae), a submersed aquatic macrophyte, is native to Asia, Australia, Europe, and Africa. This invasive species was first introduced to North America in the early 1950s in the Tampa Bay area (Schmitz et al. 1991), and dispersed rapidly to become one of the worst aquatic weeds in the U.S. (Balciunas et al. 2002). It hinders navigation, impacts water intake and delivery systems, limits recreation, out-competes native vegetation, acts as a breeding ground for mosquitoes, and destroys fish and wildlife habitats.

Management of hydrilla is typically attempted with chemical control and introduction of herbivorous fish (Hanlon et al 2000; Michel et al. 2004). Unfortunately, chemical applications can be prohibitively expensive and widespread use has led to the development of herbicide resistance to the systemic ingredient fluridone (Michel et al. 2004) and, more recently, a tolerance to endothall. The use of herbivorous fish (i.e. grass carp) has shown great promise. However, grass carp (*Ctenopharyngodon idella*) feed on a wide variety of native plant species and — while effective at removing hydrilla — in many situations, they have been proven to be highly detrimental to beneficial native plant species. In view of the previous successes with the biological control of aquatic weeds and the emergence of herbicide resistance, using biological control for the management of hydrilla has increasingly become a promising option.

Since the 1970s, funding has been directed toward implementing a viable biological control program for the management of hydrilla. The USDA-sponsored overseas surveys to identify potential agents were conducted from 1971-1976 in Pakistan, from 1985-1997 in Australia and from 1989-1995 in China and eastern Asia (Balciunas et al. 2002). Beginning in 1987, four insect species were released for the management of hydrilla, including two weevil species and two leaf-mining flies (Buckingham and Grodowitz 2004). Of these four species, only two have become established, with one species of leaf-mining fly, *Hydrellia pakistanae*, the most widespread and damaging to hydrilla. While this species has been shown to decrease photosynthesis and impact biomass, tuber and turion production, as well as decrease fragment viability (Doyle et al. 2002, 2007; Grodowitz et al. 2003; Owens et al. 2006, 2008), impact in the field has been limited. This has prompted researchers to initiate new surveys for additional hydrilla biological control agents. In addition, biological control has become a high priority since the recent discovery of herbicide resistance in hydrilla populations in Florida (Michel et al. 2004).

Locating the native range of an invasive species is a critical aspect of a biological control program. This knowledge guides exploration to areas where natural enemies are most likely to occur. Recent molecular work indicates that southern China and Southeast Asia may be the areas of greatest genetic diversity of hydrilla and therefore most likely the center of origin for the dioecious hydrilla

introduced to the U.S. (Ted Center, personal communication, USDA, ARS, Invasive Plant Research Laboratory, Ft. Lauderdale, FL).

Surveys conducted in China between 1989 and 1995 resulted in the discovery of a high number of herbivores (Buckingham 1998). Among the most promising was *Macroplea japana* (Coleoptera: Chrysomelidae), a root feeder found in Guizhou Province, Southwestern China (Buckingham 1998). In the field, however, it was recently discovered that the beetle fed on nine plant species in seven different genera, including *Vallisneria spiralis* L., *Myriophyllum verticillatum* L. and *Potamogeton malaianus* Miq. (Zhang et al. 2010). Considering how wide this host range is, it is unlikely that *M. japana* will be useful as a biological control agent for hydrilla.

Since 2006, field surveys for insect herbivores of hydrilla have been conducted in China, with the establishment of a biological control laboratory in Wuhan, Central China. Between 2006 and 2009, two new *Bagous* weevil species were found to be associated with hydrilla. *Bagous chinensis* (Coleoptera: Cucurlionidae) was collected from Liaojiachong village, Hunan Province. Unfortunately, this species was found to complete its development on a wide range of plants including *Elodea nattallii, Egeria densa, Vallisneria natans,* and *Hydrocharis morsus-ranae*, indicating it is not suitable for use as a biocontrol agent in the U.S. The most important finding in 2008 – 2009 was a second *Bagous* weevil (designated *Bagous* sp. 1 in this report) discovered in the Li River in Guangxi Province. Though we have little information on the host range of *Bagous* sp. 1, these new findings indicate that the diversity of weevils feeding on hydrilla is greater than previously thought.

Despite many years of hydrilla exploration in Asia, the regions where the U.S. dioecious hydrilla biotype is native (in Southern China and Southeastern Asia) have yet to be thoroughly explored for potential biological control agents. The main objective of this project is to continue and expand surveys on hydrilla throughout the entire growing season in unsurveyed regions of southern China. The provinces of Hubei, Hunan, Sichuan, Guizhou and Guangxi will be surveyed on a regular basis; i.e., four — and up to ten — field sites will be visited two to three times during the growing season.

## Project update: field collections and lab rearing

**Survey Areas.** Due to the heavy flooding in Southern China from May to early July 2010, field surveys were postponed until late July. Since then and continuing through late November 2010, surveys were conducted for hydrilla herbivores in Hubei, Anhui, Jiangxi and Yunnan Provinces in China (see map, Fig. 1). Although Xishuangbanna, a tropical area in Yunnan, was visited in mid-October, no samples were collected due to the heavy flooding in that area. Hydrilla samples were collected at a total of 21 sites during 2010 (Table 1).

**Sample Processing:** After collection, hydrilla samples were placed in a cooler/refrigerator then transported to the lab. Any insect damage was carefully examined and recorded as were any insect stages found. Following visual inspections, the samples were placed into Berlese funnels to allow for further extraction of organisms present on the samples.

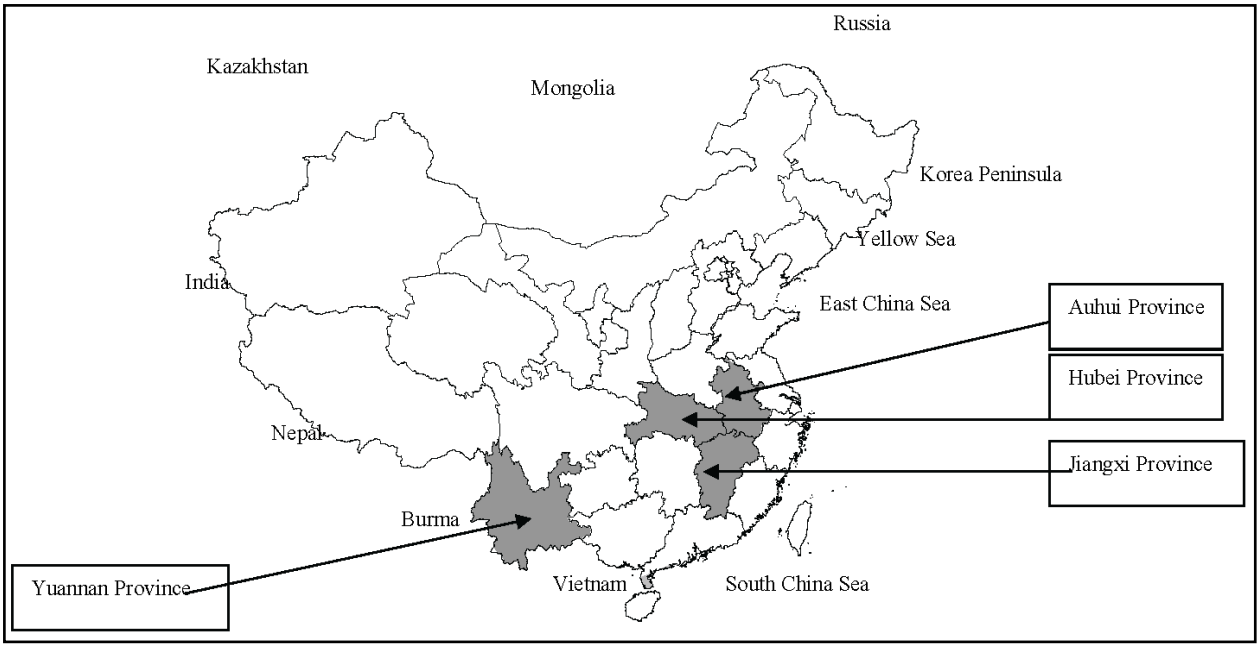


Figure 1. Chinese provinces surveyed in 2010 for potential biocontrol agents of hydrilla.

**Table 1 Field sites and insect collections from hydrilla samples during July-November, 2010.***

| Province | County | Site name | Location | Specimens Collected |
|---|---|---|---|---|
| Hubei | Xingshan | Yanjiawan | N31°19′07″<br>E110°44′10″ | No insects |
| | Fangxian | Mojiagou | N32°03′40″<br>E110°41′41″ | Parapoynx sp.:17L<br>Chironomidae: 8L |
| | Yuxian | Hujiaying | N32°46′22″<br>E110°15′47″ | Parapoynx sp.:4L |
| | Shiyan | Bolin | N32°39′21″<br>E110°37′31″ | Parapoynx sp.: 7L<br>Chironomidae: 13L |
| | Danjiangkou | Tuguanya | N32°26′12″<br>E111°22′06″ | Parapoynx sp.: 13L<br>Chironomidae: 6L,<br>Hydrellia:3L |
| | Suizhou | Huangtan | N31°46′27″<br>E113°07′37″ | Bagous sp: 2A, 6L<br>Unkown weevil, 1A<br>Parapoynx sp.: 73L, Chironomidae: 136L |
| | Suizhou | Changgang | N31°33′21″<br>E112°57′50″ | Parapoynx spp.: 7L,<br>Chironomidae: 11L |
| | Guangshui | Pinglin | N31°24′44″<br>E113°36′22″ | Hydrellia:19L,1A<br>Chironomidae: 16L |
| | Guangshui | Maping | N31°33′05″<br>E113°33′02″ | Parapoynx sp.: 7L<br>Chironomidae: 43L |
| | Suizhou | Xihe | N31°40′55″<br>E113°28′10″ | Parapoynx sp.: 2L |
| | Suizhou | Anju | N31°44′37″<br>E113°11′40″ | Parapoynx spp.: 10L, Chironomidae: 3L<br>Hydrellia:40L |
| | Macheng | Muzidian | N31°15′01″<br>E115°25′58″ | Parapoynx sp.: 245L |
| Anhui | Susong | Suishicun | N30°04′05″<br>E116°11′26″ | Parapoynx sp.: 5L<br>Hydrellia:3L |
| | Taihu | Xiaochi | N30°33′15″<br>E116°27′15″ | Parapoynx spp.: 57L |
| | Qianshan | Guanhelu | N30°38′10″<br>E116°34′455″ | Parapoynx sp.: 3L<br>Chironomidae: 5L |
| | Huoshan | Dongcheng | N31°23′55″<br>E116°20′08″ | Parapoynx sp.:7L, Chironomidae:10L |
| | Huoshan | Nayuan | N31°23′53″<br>E116°16′27″ | No insects |

**Table 1 Field sites and insect collections from hydrilla samples during July-November, 2010.***

| Province | County | Site name | Location | Specimens Collected |
|---|---|---|---|---|
| | Qimen | Jinzipai | N29°50′49″<br>E117°46′49″ | Parapoynx sp.:15L<br>Hydrellia:1L |
| | Huangshan | Tunxi | N29°42′28″<br>E118°19′01″ | Parapoynx sp.:6L<br>Hydrellia:1A |
| | Huangshan | Sankou | N30°17′23″<br>E118°13′58″ | Parapoynx sp.:5L, Chironomidae:3L |
| | Yixian | Xidi | N29°53′42″<br>E117°57′21″ | Parapoynx sp.: 5L<br>Chironomidae: 2P<br>Hydrellia:1L |
| Jiangxi | Wuyuan | Ziyang | N29°15′27″<br>E117°50′59″ | Parapoynx sp.: 1L<br>Hydrellia:4L |

* Number after the insect species indicates the total number of individuals collected from each site. L: larva; A: adult

**Insects Collected.** The generalist herbivore *Parapoynx* (Lepidoptera: Crambidae) was dominant at almost all the sites (Fig. 2). In some cases, it occurred at extremely high densities. For example, 245 larvae were collected from one site at Muzidian in Macheng county of Hubei Province. Since these species usually feed on a great number of aquatic plants, it has no use as a biocontrol agent.

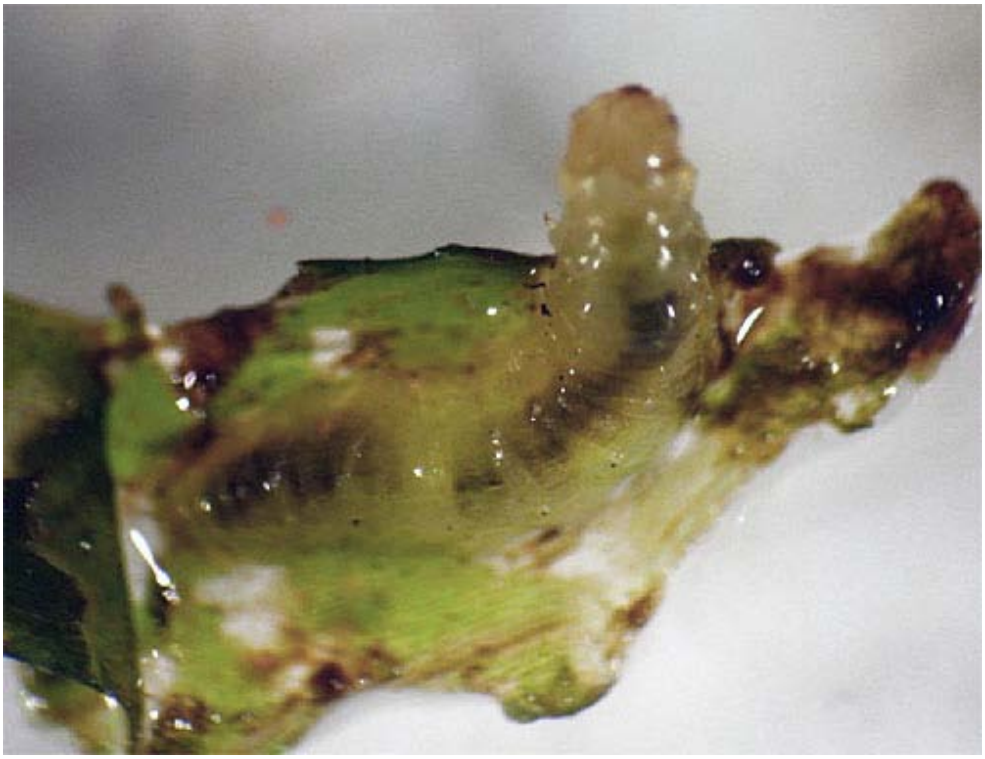

Figure 2. Larva of Parapoynx sp., a commonly collected generalist herbivore found associated with hydrilla throughout China. Larvae live, feed, and develop in cases cut from hydrilla leaves.

Figure 3. Example of a type of midge larva commonly collected from hydrilla throughout China.

Midges (Diptera: Chironomidae) (Fig. 3) and leaf-mining flies in the genus *Hydrellia* (Diptera: Ephydridae; Figs. 4-5) were also commonly collected at many of the sites. Most of these are similar to those identified in previous surveys conducted during the 1990s and during recent surveys in 2006-2008 and are of little or no interest.


Figure 4. Commonly collected *Hydrellia* larva (a) and adult (b).

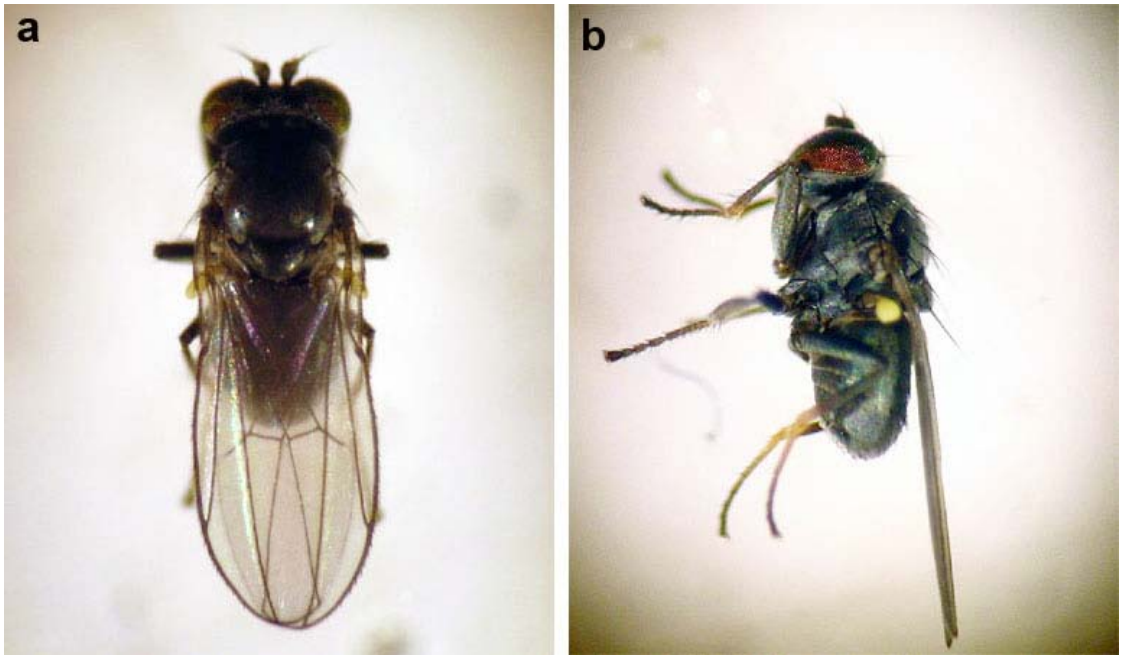


Figure 5. *Hydrellia* sp. 1 collected near Pinglin Town, Guangshui City, Hubei Province and Tunxi, Huangshan City, Anhui Province showing dorsal (a) and lateral (b) views.

The most interesting collection from the surveys was a possible new *Bagous* species (Coleoptera: Curculionidae) (designated *Bagous* sp. 2 in this report) from a river near Huantan Town, Suizhou City, Hubei Province (Figure 6). Two adults and six larvae of this species were collected from hydrilla at Suizhou, Hubei Province. When brought into the laboratory, both adults and larvae were found to feed on hydrilla leaves. Larvae of *Bagous* sp. 2 pupated along the hydrilla shoot with the pupal cocoon surrounded by algae (Fig. 7). Though *Bagous* sp.2 appears similar to *Bagous* sp. 1, their life cycles are apparently different, which suggests this collection may represent a new species. The two weevil species identified in previous surveys pupate along the shore in drying or dried soils. This is an important finding since *Bagous* pupation on hydrilla under water has never been identified previously.

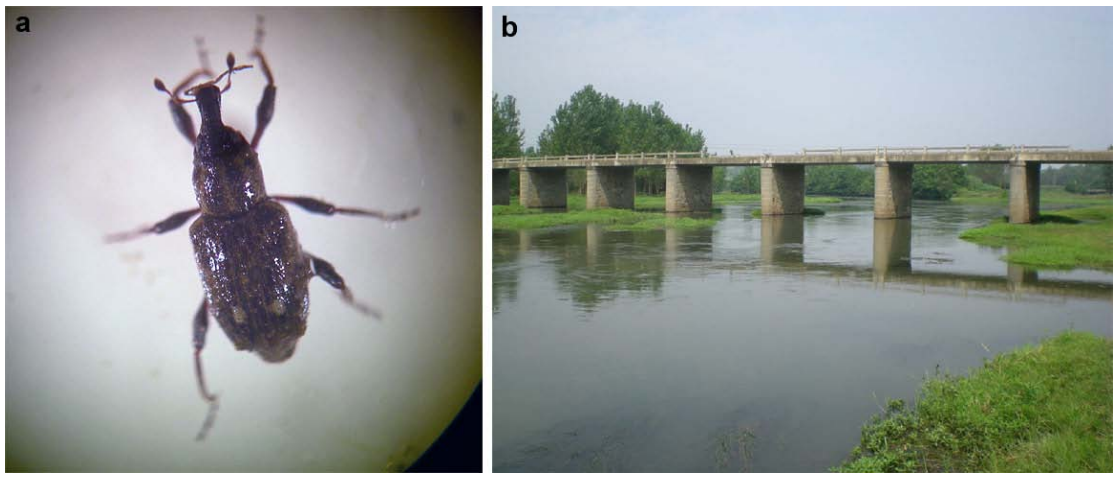


Figure 6. *Bagous* sp. 2 (a) and collection site (b) near Huantan Town, Suizhou City, Hubei Province.

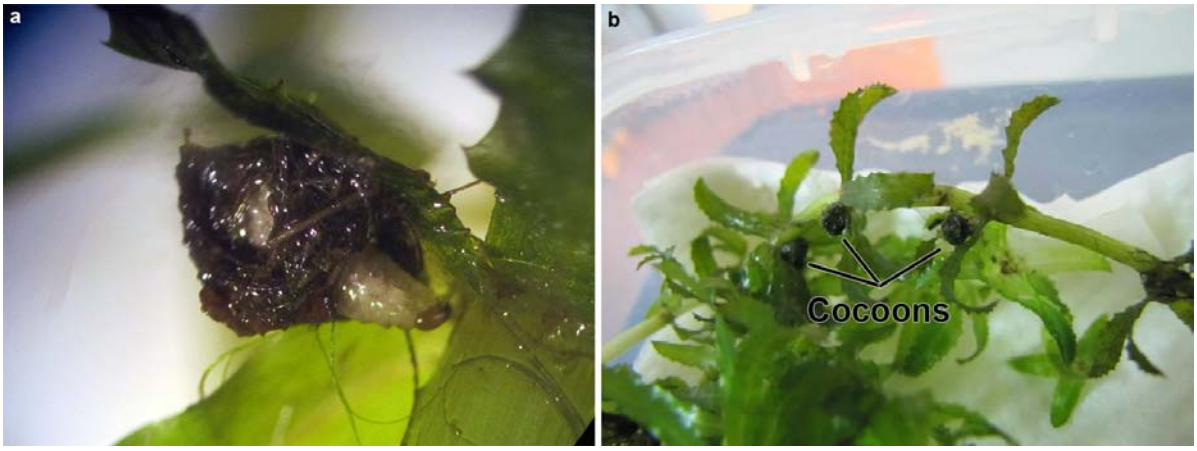


Figure 7. A late instar larva surrounded by algae (a) and cocoons along hydrilla shoot (b).

## Ongoing work and future plans:

**Field surveys:** Pending funding, additional surveys are planned for the coming months and throughout 2011. Extensive field surveys to Yunnan, Sichuan and Guizhou Provinces are in progress with more planned in the coming months. At least four and up to ten field sites in each province will be visited. Since all the sites are located in the tropical or sub-tropical range, winter surveys are possible.

**Lab rearing of new Bagous weevil:** Laboratory rearing of *Bagous* sp.2 is continuing in the Wuhan laboratory. The colony will continue to be extended over the next few months. During the rearing process, observations will be made on its biology and ecology. If more offspring are obtained, host range tests will be initiated.

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