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Diasemiopsis ramburialis (Duponchel) (Lepidoptera, Pyralidae *s. l.*, Spilomelinae) in Iran: first record for the country and first host plant report on water fern (*Azolla filiculoides* Lam., Azollaceae)

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Abstract. During a survey at the Rice Research Institute of Iran (RRII, Rasht, Guilan) for potential biocontrol agents of water fern, *Azolla filiculoides* Lam. (Pteridophyta: Azollaceae), larvae of *Diasemiopsis ramburialis* (Duponchel) (Pyralidae *s. l.*, Spilomelinae) were discovered feeding on water fern. Larvae were found to cause serious feeding damage on leaves of water fern in the laboratory. The biology, life cycle, and the morphology of all stages of this species are described and illustrated for the first time. This is also the first record of this cosmopolitan species in Iran. We report water fern as a host for *Diasemiopsis ramburialis*; until now the host plant of *D. ramburialis* was unknown.

Introduction

Diasemiopsis ramburialis (Duponchel) is a snout moth in the subfamily Spilomelinae, the most species-diverse subfamily of Pyralidae *sensu lato* (or Crambidae, depending on authors, see Regier et al. 2012). Diagnostic characters for this subfamily are: head without chaetosemata, male hindwing without subcostal retinaculum, tympanal organs with a bilobed praecinctorium, projecting fornix tympani, and pointed spinula, male genitalia without gnathos, and corpus bursae of female genitalia usually without rhomboid signum (Minet 1982; Regier et al. 2012).

Diasemiopsis was described by Munroe (1957) with *Hydrocampa ramburialis* Duponchel, 1833 as type species. Only one other species, *D. leodocusalis* (Walker, 1859), described from the United States of America, is currently assigned to this genus (Nuss et al. 2015). The full synonymy of *D. ramburialis* is given by Nuss et al. (2015).

Adults of *D. ramburialis* are grey or brown, with two broad zigzagging white lines across each wing. They measure 17–22 mm in wingspan (n=20) (Fig. 1). Described from France (Corsica), this species has been reported from Africa (e.g. Maes 2004), the Seychelles (Aldabra Atoll) (Shaffer and Munroe 2007), Réunion (Guillermet 2009), Europe (Karsholt and Nieukerken 2013), Puerto Rico (Möschler 1890; Schaus 1940; Patterson 2015), Austral Islands (Rapa) (Clarke 1971), French Polynesia (Tahiti) (Oboyski 2015), Australia (Shaffer et al. 1996), Taiwan (Wang and Speidel 2000), and India (Kirti and Sodhi 2001). Clarke (1971) reported it as a cosmopolitan species and illustrated the habitus and male and female genitalia (see also Guillermet (2009) and Slamka (2013) for additional illustrations). Regarding records from the New World, Munroe (1957) mentioned

Figure 1. Adult of *Diasemiopsis ramburialis*.

that they actually refer to *D. leodocusalis* (Walker). Maes (2004) added that the species seemed to be linked to swampy areas. There is no record of host plant or any description of the immature stages available for *D. ramburialis.*

Azolla filiculoides Lam. (Azollaceae) is considered a major aquatic weed in several countries (Zimmerman 1985). It is a small-leafed floating aquatic fern native to the tropics, subtropics, and warm temperate regions of Africa, Asia, and the Americas (Costa et al. 2009). It is one of the world's fastest growing aquatic macrophytes, with a doubling time of only 2–5 days in biomass (Zimmerman 1985; Taghi-Ganjiet al. 2005). Some species of *Azolla* provide various benefits such as a source of organic nitrogen, soil improvement and nutrient availability, weed suppression, and as food for livestock, chicken, ducks and fishes (Anonymous 1987; Ferentinos et al. 2002). However, some of them, *A. pinnata* (R. Br.) and *A. filiculoides* in particular (e.g. Barreto et al. 2000), are considered major weeds in South Africa, Europe, and New Zealand (Hill 2003; Bodle 2008; Sadeghi et al. 2013) and *A. filiculoides* is an alien species in Iran (JICA 2005; Delnavaz and Azimi 2009).

In Iran, this weed invades rice fields and aquatic natural habitats, such as the Anzali (Delnavaz and Azimi 2009; Sadeghi et al. 2013) and Amir-kelayeh wetlands (Fig. 2) of the Guilan province. These wetlands have ecological importance for breeding, wintering, and survival of many species of birds, fish, and microorganisms (Khoshechin 1993; Naddafi et al. 2005; Charkhabi and Sakizadeh 2006; Moradinasab et al. 2012). Infestations of *A. filiculoides* reduce the quality of the water used for agricultural and human use, and simplify local aquatic food webs (Hill 1998). Control options for the water fern in Iran are limited because mechanical methods are impractical and there are no registered herbicides for aquatic ecosystems. This situation stimulated the use of biological control as a sustainable strategy for the long term management of *A. filiculoides* (Richerson and Grigarick 1967; McConnachie et al. 2003). *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae), a weevil native to the USA, had a huge impact on *A. filiculoides* as a biological control agent. This weevil controlled water fern in Africa, USA, and other regions (Hill et al. 2008; Partt

Figure 2. A waterway near Amir-kelayeh wetland covered with water fern.

et al. 2013). However, the identification of other active herbivores on *Azolla* as biotic resistance factors and competitor species with the main biological control agent was found to be desirable because other species may have negative or positive effects on the establishment of the main biological control agent. We conducted surveys in rice fields for two years due to a lack of information on local herbivores attacking *A. filiculoides* in Iran. The specific objective of this paper is to report the life history of *D. ramburialis* attacking *A. filiculoides* in Iran.

Material and methods

Rearing: Laboratory colonies were established by collecting larvae from water fern located on waterways and experimental rice fields at the Rice Research Institute of Iran (RRII) (N37°12’22.2”, E049°38’40.7”, 80 masl) from September to November 2013 and 2014.

Larvae were kept in 14 cm diameter petri dishes filled with water fern and 100 cc of distilled water. Petri dishes of the same size were used to cover the dishes to provide more space for larvae and newly emerged adults. Water was changed every 4 days and water fern was added if needed. The petri dishes were changed every 10 days to prevent the growth of fungi and bacteria.

Upon emergence, a pair, a male and female, were released into 14 cm covered petri dishes. To provide more space for the moths, we set up three 6 cm petri dishes each filled with 10 cc of distilled water and 5 g of water fern. The 6 cm petri dishes were changed daily and placed in an incubator until egg hatching. The incubator was set at 25–27 °C and 16: 8h (L: D) photoperiod. Thereafter, first or second instar larvae were placed in 6 cm petri dishes provided with 10 cc of water and 5 g of water fern in groups of one, two, and three individuals in each dish. Since the larvae had wandering habits, each 6 cm petri dish was placed in a 14 cm covered petri dish. The 6 cm petri dishes were changed every three days and water fern was added if necessary. Moth colonies were inspected daily and all activities, including egg hatching, larval feeding period, pupation, and emergence of adults were recorded.

Identification: Twenty adults were used for preliminary identification based on wing venation and other morphological characters. Dissection of both male and female genitalia (Figs 7–9), were made following Landry (2003) and Lee and Brown (2009). Dissections were conducted at the RRII laboratory and sent to B. Landry for final determination. The specimens were identified as *Diasemiopsis ramburialis* (Duponchel, 1834).

Field observations: During 2013 and 2014, feeding damage observations by *D. ramburialis* on water fern and other potential host plants were conducted in the waterways and experimental rice fields located at RRII.

Results

Eggs: About 1 mm in diameter, globular, opaque to pale orange (Fig. 3), they turn to dark orange or reddish brown before hatching. They are laid singly or in groups of two or three on water fern leaves or smooth surfaces of other material (Petri dishes) near the food plant. Development time at 25–27 °C was approximately 4 days.

Larva: Newly hatched larvae are dark orange to reddish brown with a black head (Fig. 4), about 1.5 mm in length, and have sparse setae on the body. As the larva develops, its colour becomes darker, the setae increase in length, and dark spots appear at the base of the setae. The last instar larva is greenish brown (Fig. 5) and about 18 mm in length. There are black plates on each thoracic and abdominal segment. The prothoracic shield is pigmented laterally and unpigmented medially and there are four dorsal, two lateral, and two ventral pinacula on the meso- and metathorax. On abdominal segments I–VII there are four dorsal, two lateral, and two ventral pinacula on each segment. On abdominal segment VIII there are three dorsal pinacula in a triangular pattern of two rows: two pinacula in first row and one in second row; this segment has two lateral and two ventral pinacula as well. Abdominal segments IX and X respectively have one dorsal pinaculum and one dorsal and one lateral pinaculum. There is little space between these pinacula and these areas appear as three longitudinal bands. The development time of 100 larvae at 25–27 °C was 14 to 15 days.

Pupa: Pupation usually occurred on dried water fern or mud on the side of the dishes. The length of pupa is approximately 7–9 mm and the colour turns from yellowish brown to dark brown during development (Fig. 6). The development time of 50 pupae at 25–27 °C was 7 days.

Adults: The habitus and male and female genitalia are illustrated in Figs 1 and 7–9. The longevity of adults at 25–27 °C with and without a sugary solution (10% honey) was <30 days and 14 days, respectively (at least 30 adults were followed under each treatment).

Symptoms of damage on host plants: Larvae of *D. ramburialis* are phyllophagous. We have observed that newly hatched larvae build shelters by binding leaves together with silk and remain hidden in their shelters when they are not actively feeding. When larvae mature, they leave their shelter and wander on water fern leaves in silky tunnels filled with frass (Figs 10 and 11). Feeding damage by larvae facilitated the infection of water fern with bacteria and fungi. Egg laying occurred during the entire lifetime (ca. 30 days) and the number of eggs per day varied considerably.

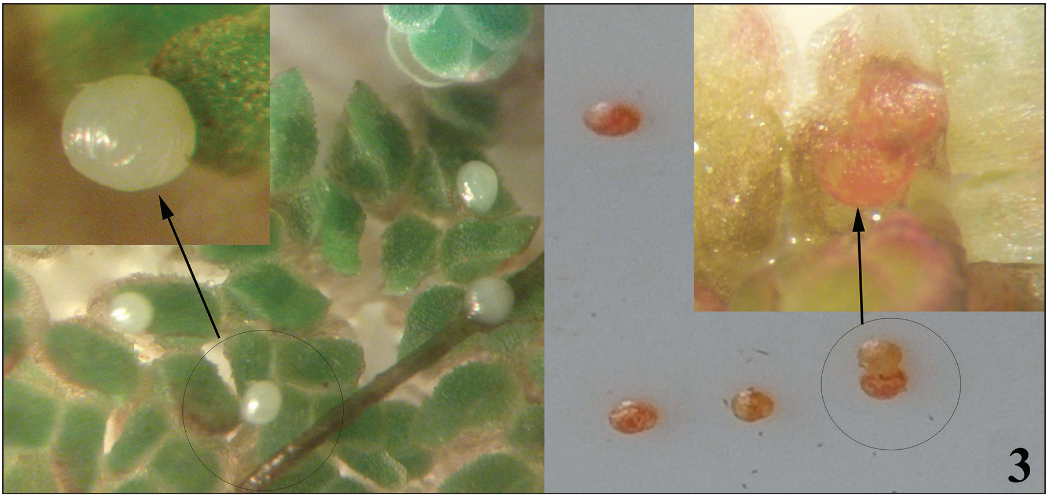
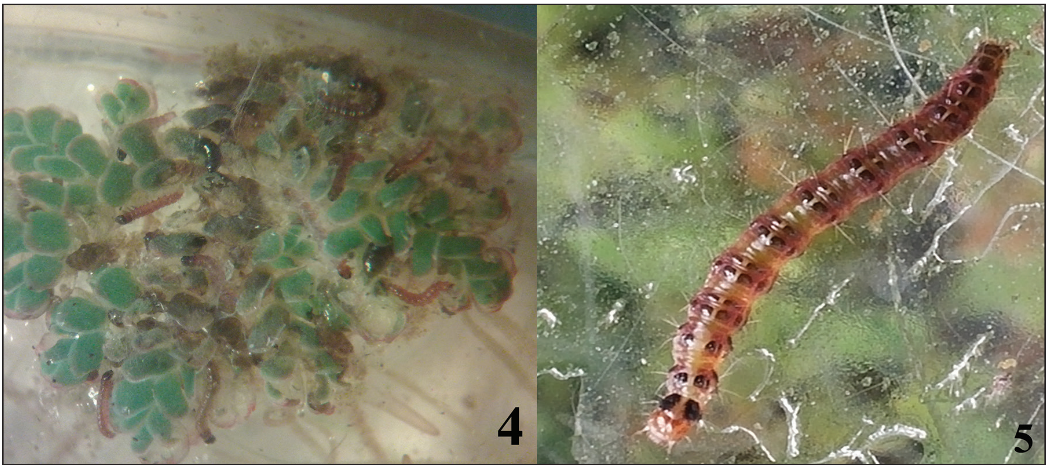


Figure 3. *Diasemiopsis ramburialis* eggs (Right: Infertile, Left: Fertile).



Figures 4, 5. *Diasemiopsis ramburialis* larvae **4.** First instar, **5.** Last instar.

Discussion

In northern regions of Iran, aquatic ecosystems such as stagnant waters, ponds, ditches, canals or paddy fields may be covered seasonally by *Azolla* (Delnavaz and Azimi 2009; Sadeghi et al. 2013) in association with other floating aquatic plants including *Lemna minor* L. (duckweed: Lemnaceae), *Trapa* sp. (water caltrop: Trapaceae), *Wolffia* sp. (water meal: Lemnaceae), or *Salvinia* sp. (Salviniaceae), and mud-rooting species such as *Ceratophyllum demersum* L. (hornwort: Ceratophyllaceae), *Ludwigia palustris* (L.) Elliott (water purslane or water primrose: Onagraceae), and *Polygonum arenastrum* Boreau (knotweed: Polygonaceae) (Delnavaz and Azimi 2009; Kannaiyan and Kumar 2006; Mozafarian 2007). *Azolla* is not native to the northern region of Iran and was introduced in 1986 (Delnavaz and Azimi 2009). However, many of the above-mentioned aquatic plants are native in this region (Mozafarian 2007) and many insects use them as food plants.

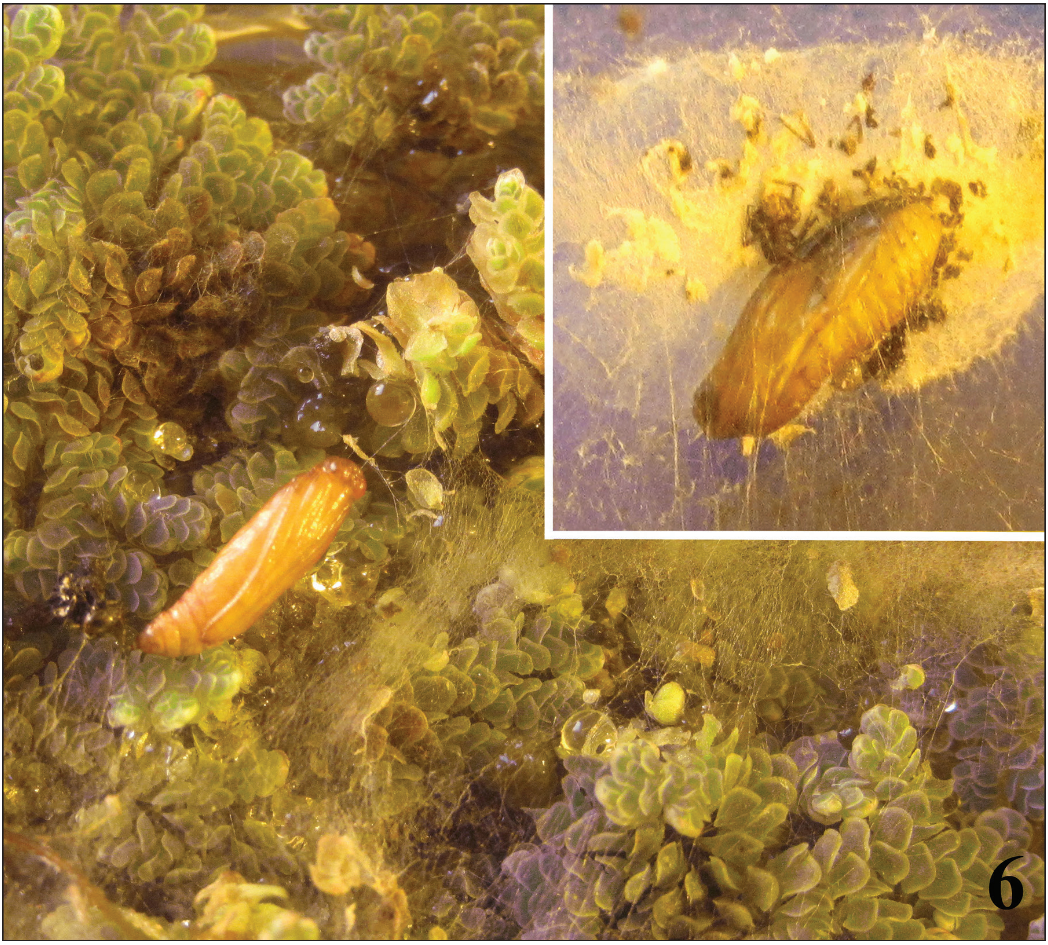
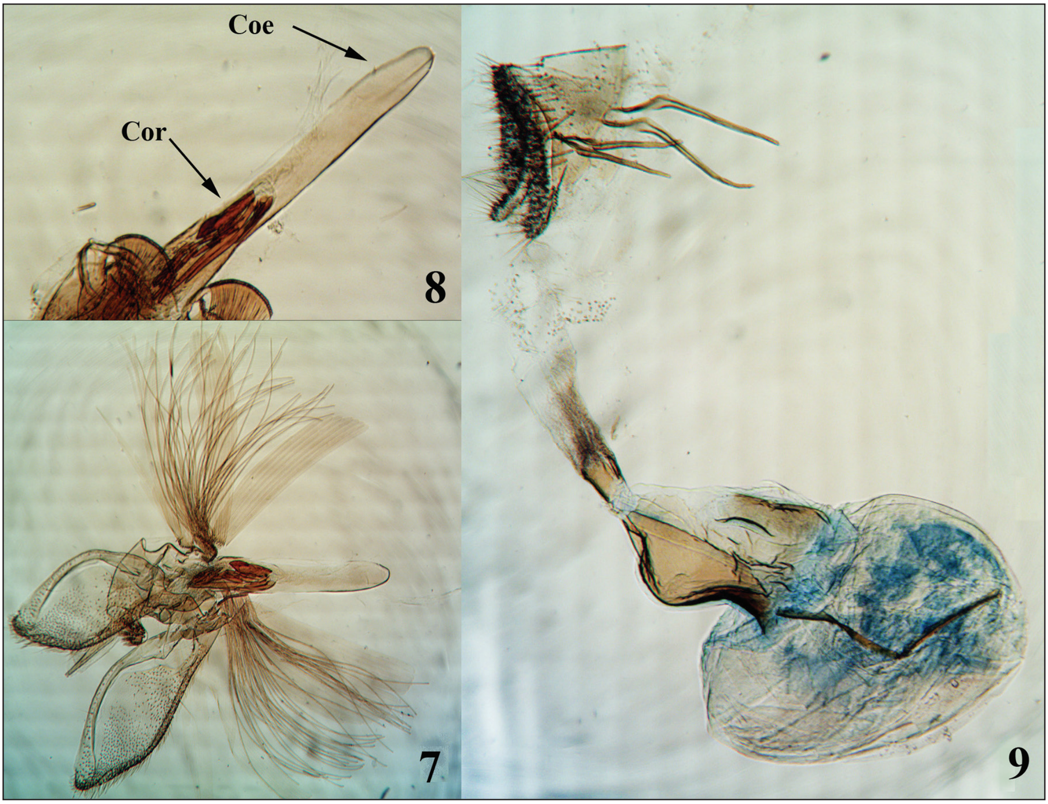


Figure 6. *Diasemiopsis ramburialis* pupa (Magnified: Mature pupa).

During the present study we investigated the activity of *D. ramburialis* on *Azolla* in rice fields. In Iran, *D. ramburialis* adults start their activity in July but they are most active in rice fields from September to late October. In these areas, this is almost the end of harvesting time and the end of the summer. The temperature gets slightly cooler and fields become half-dried, which eases harvesting. The reasons for the increase in the activity of adults in rice fields at the end of the summer, when water fern is getting dry due to water stress, are not clear.

Water depth could be a restricting factor for the activity of *D. ramburialis*. It is possible that pupae are sensitive to high water depth and are not able to survive under submerged conditions in rice fields during the cultivation season. In addition, culturing operations in rice fields disturb the water fern layer constantly and destroy pupae. However, after harvesting, since water fern remains undisturbed and fields become dry, *D. ramburialis* can increase its populations.

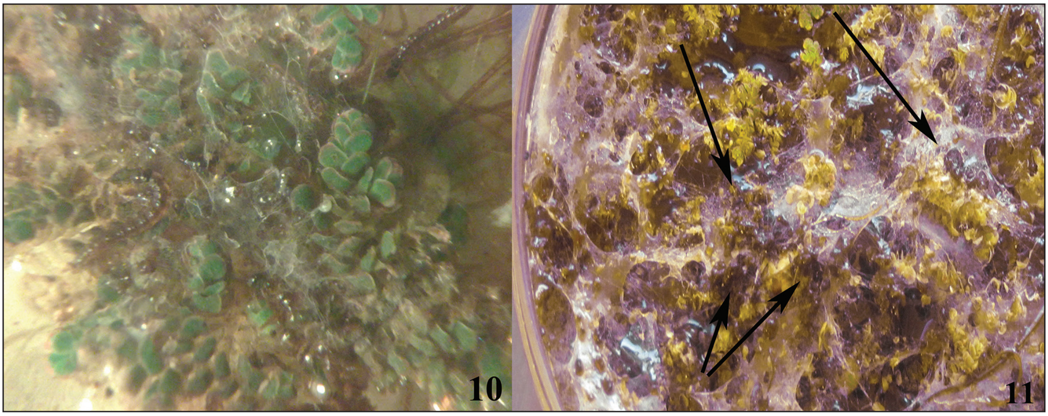
On the other hand, rice fields are an anthropogenic agricultural ecosystem in which thermal conditions can differ significantly from those of natural aquatic habitats. Discharge of heated water, artificial mixing of thermal strata, impoundment, diversion, regulation of water level



Figures 7–9. *Diasemiopsis ramburialis* genitalia. **7.** Male genitalia with phallus to the right and uncus+tegumen underneath right (top) valva. **8.** Phallus (Cor = Cornuti; Coe = Coecum). **9.** Female genitalia.

and flow, and canopy opening in riparian zones, through harvest or grazing, severely modify the thermal environment for aquatic species (Ward and Stanford 1982). Therefore, in wetlands without the disturbance of agricultural processes and chemical compounds *D. ramburialis* could behave differently.

Our study is the first to record a host plant for *D. ramburialis* and the genus *Diasemiopsis* because the host plant of the North American *D. leodocusalis* is still unknown. However, there are other Spilomelinae, such as the salvinia stem borer *Samea multiplicalis* (Guenée), that feed on several Salviniaceae and *Azolla*. This moth has been known as a potential biocontrol agent for *Salvinia molesta* DS Mitchell (Pelli et al. 2008). In host range tests Knopf and Habeck (1976) stated that this moth has three main host plants in Florida (USA): *Azolla caroliniana*, *Pistia stratiotes*, and *Salvinia rotundifolia*. However, this moth has not been reported on these host plants from Iran or the Palaearctic and African regions. Although we briefly studied the biology of *D. ramburialis* as a probable biotic resistance factor for water fern, many other important biological aspects such as life span in natural habitats, population growth parameters, host range, and host preference remain unclear. Also, we could not find any specific parasitoid or predator for this moth although there are many generalist predators and parasitoids active in rice fields (Ooi and Shepard 1994; Shepard et al. 1987) that could affect *D. ramburialis*.



Figures 10–11. Feeding activity and webbed shelters of *D. ramburialis* larvae. **10.** First instar larva making feeding shelter with silk. **11.** Shelter full of frass after larval feeding.

Conclusion

Environmental conditions of northern regions of Iran, in addition to agricultural activity, put native living organisms under various kinds of stress and shape them into trying to adapt to different conditions. However, in comparison to the long period of adaptation of native species, some exotic organisms such as water fern have adapted themselves to local conditions in a shorter period of time and have become dominant in some areas (Delnavaz and Azimi 2009; Sadeghi et al. 2013). This invasion puts native plants under pressure and the insects that feed on native plants either have to use new plants as a food resource or die of hunger. Despite a rich fauna of aquatic and semi-aquatic insects, water fern biotic resistance factors in Iran are still poorly known. We started our surveys on water fern biotic resistance factors in Iran's northern region rice fields in 2013 and this is the first report of our results. More studies are under way to uncover important facts on the biotic resistance factors of this non-native invasive weed in northern regions of Iran.

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