Insect Herbivores Associated with Nymphaea mexicana (Nymphaeaceae) in Southern United States: Potential Biological Control Agents for South Africa

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Insect herbivores associated with *Nymphaea mexicana* (Nymphaeaceae) in southern United States: potential biological control agents for South Africa

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Abstract

Nymphaea mexicana Zuccarini (Nymphaeaceae) (Mexican waterlily) is an emergent floating-leaved aquatic plant from the southeastern USA that is invasive in South Africa. In invaded waterbodies this plant restricts water movement, increases siltation, decreases recreational activities, and can deplete water oxygen levels, which in turn negatively impacts aquatic fauna. Currently there are no chemical, mechanical, or biological control programs in place for *N. mexicana* in South Africa, but the sustainability of biological control makes this the most desirable option. Field surveys for potential biological control agents were conducted in the native range of *N. mexicana* in Florida, Louisiana, and Texas from Aug to Oct 2018. Leaves, stems, flowers, and roots of *N. mexicana* were searched for insect herbivores by hand and using Berlese funnels. Insects were prioritized for use as biological control agents by considering the extent and type of feeding damage, field host range, and incidence (percentage of sites in which each species was found). In total, 15 confirmed species were found feeding on *N. mexicana*, and some taxa were identified only to family level. Incidence coverage estimator mean, MMRuns, Chao 2 mean, and Chao 2 upper 95% CI species accumulation estimators predicted that between 2 and 5 species were missed during the surveys. Based on field observations, *Bagous americanus* LeConte (Coleoptera: Curculionidae), and *Megamelus toddi* Beamer (Hemiptera: Delphacidae) were prioritized. Host specificity trials will be conducted to determine whether these insects may be used as biological control agents of *N. mexicana*.

Key Words: field surveys; Mexican waterlily; yellow waterlily; invasive alien plant

Resumen

Nymphaea mexicana Zuccarini (Nymphaeaceae) (Mexican waterlily) es una maleza acuática emergente nativa del sureste de Estados unidos, y considerada invasiva en África del Sur. En humedales invadidos, esta planta limita el movimiento de agua, incrementa sedimentación, decrece el valor recreacional, y puede reducir los niveles de oxígeno líquido, lo cual impacta negativamente la fauna acuática. No existe programas de control químico, mecánico o biológico para *N. mexicana* en África del Sur, pero la sostenibilidad del control biológico hace esta opción las más deseable. Desde Agosto a Octubre del 2018 se realizaron muestreos de campo para recolectar agentes de control biológico en el rango nativo de *N. mexicana* en Florida, Luisiana, y Texas. Insectos herbívoros de hojas, tallos, flores, y raíces de *N. mexicana* fueron buscados a mano o con embudos de Berlese. Insectos fueron priorizados para el uso como agentes de control biológico dependiendo del tamaño y tipo de daño, el rango de hospederos de campo, y su densidad de campo (porcentaje de sitios en los cuales la especie fue encontrada). Quince especies fueron encontradas alimentándose de *N. mexicana*, y posiblemente más están presentes debido a que taxones fueron identificados al nivel de familia. Los estimadores de especies acumulados media estimador de cobertura de incidencia, NMRuns, media Chao, y Chao por arriba del 95%, predijeron que entre dos y cinco especies no fueron encontradas durante los muestreos. Basado en observaciones de campo, *Bagous americanus* LeConte (Coleoptera: Curculionidae), y *Megamelus toddi* Beamer (Hemiptera: Delphacidae), fueron priorizados. Ensayos de especificidad serán realizados para determinar si esos insetos pueder se usados como agentes de control biológico de *N. mexicana*.

Palabras Clave: muestreos de campo; Ninfa Mexicana; Apapatla; planta invasive

Biological control is a proven method for managing invasive alien plant populations (McFadyen 1998; Zachariades et al. 2017). In South Africa, many of the worst floating aquatic weeds are under complete biological control (Hill & Coetzee 2017). However, this niche is now vulnerable to invasion by submerged plants, such as *Egeria densa* Planch. (Hydrocharitaceae) (Brazilian water weed) and *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae) (hydrilla), as well as emergent invasive alien species such as *Sagittaria platyphylla* (Engelm.) J.G. Sm. and *Sagittaria latifolia* Willd. (Alismataceae) (delta arrowhead); *Lythrum salicaria* L. (Lythraceae) (purple loosestrife), *Nasturtium offi-*

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cinale W.T. Aiton. (Brassicaceae) (watercress), *Iris pseudacorus* L. (Iridaceae) (yellow flag), and *Hydrocleys nymphoides* (Humb. & Bonpl. ex Willd.) Buchenau (Alismataceae) (water poppy) (Coetzee et al. 2011; Hill & Coetzee 2017). Furthermore, floating weeds such as *Salvinia minima* Baker (Salviniaceae) (salvinia) and *Azolla cristata* Kaulf. (Azollaceae) (Mexican azolla) also have been recorded in their early stages of invasion (Hill & Coetzee 2017), as well as the rooted floating Mexican water lily, *Nymphaea mexicana* Zucc. (Nymphaeceae). Due to their potential invasiveness, most of these species are now targets for biological control.

Nymphaea mexicana (Mexican waterlily or yellow waterlily) is a hardy water lily native to the southeastern USA and Mexico (Conard 1905 cited in Capperino & Schneider 1985), but has become invasive in numerous regions of the world, including New Zealand, India, and Spain, in addition to South Africa (Johnstone 1982; Garcia-Murillo 1993; Henderson 2010; Newfield & Champion 2010; Hussner 2012; Shah & Reshi 2012). Due to the aesthetic appeal of this plant, many introductions have occurred through the ornamental nursery trade, and its rapid growth has allowed *N. mexicana* to spread rapidly and invade aquatic ecosystems, thereby altering ecosystem function (Capperino & Schneider 1985; Marcos 1985). *Nymphaea mexicana* is classified as a category 1b invasive weed in South Africa according to the National Environmental Management: Biodiversity Act (No. 10 of 2004). Under this category, trade or planting is prohibited and this species must be controlled, and where possible, removed and destroyed.

The biology of this plant makes mechanical removal difficult because, unlike floating weeds, the roots must be removed from submerged soils or it will resprout from rhizomes and stolons (Johnstone 1982). The cost and environmental impacts of chemical and mechanical control thus make biological control the most desirable, sustainable, and effective means of controlling this plant in the long-term, yet no phytophagous insect species have been investigated for its control. Therefore, a biological control program for the management of *N. mexicana* in South Africa was initiated in 2016, necessitating field surveys in the region of origin to select suitable agents. Prioritization of potential agents can be achieved by (a) sampling in regions that are climatically similar to the invaded range, because the natural enemies found there should be adapted to those conditions and are thus more likely to establish (Robertson et al. 2008), and (b) sampling from populations of the invasive plant that are genetically similar to those in the invaded range (Sheppard et al. 2003, but see Van Klinken et al. 2003). Additionally, criteria for prioritization include: level and mode of feeding damage, for example, natural enemies that damage vascular and mechanical support tissues are more likely to be effective biological control agents (Harris 1973); incidence, that is the percentage of sites at which each species is recorded; and field host range (Olckers 2000; Paterson 2010). Hence, this study aimed to identify the phytophagous insect species that feed on *N. mexicana* in its native range, and prioritize species based on feeding damage and host range as potential biological control agents of *N. mexicana*.

Materials and Methods

MAXENT MODELLING AND SURVEYS

Surveys for natural enemies took place in the southeastern USA and were focused mainly in Florida due to the abundance of sites, but also in Louisiana and Texas. Sites within the native range of *N. mexicana* were selected using MaxEnt modelling (Phillips et al. 2004, 2006) to match regions with a similar climate to areas in South Africa infested with *N. mexicana* (Goolsby et al. 2006). Locality data were obtained from the Global Biodiversity Information Facility (GBIF 2019), Southern African Plant Invaders Atlas on the Botanical Database of Southern Africa online website (Henderson 2007; South African National Biodiversity Institute 2016), and records from field surveys (Naidu 2018). Locality coordinates were filtered by removing invalid points (e.g., points found on land in dry areas are inappropriate for aquatic plants), and the model was generated based on coordinates from the native range and invaded sites in South Africa. Bioclimatic predictor variables were downloaded from the WORLDCLIM database (Hijmans et al. 2005) (http://www.worldclim.org/), and the following predictor values were selected from 'bioclimatic variables' (BIO x): mean annual temperature (BIO 1); mean diurnal range (mean of monthly [maximum temperature − minimum temperature]) (BIO 2); isothermality (BIO 3); temperature seasonality (BIO 4); maximum temperature of warmest month (BIO 5); minimum temperature of coldest month (BIO 6); temperature annual range (BIO 7); mean temperature of wettest quarter (BIO 8); mean temperature of driest quarter (BIO 9); mean temperature of warmest quarter (BIO 10); mean temperature of coldest quarter (BIO 11); and annual precipitation (BIO 12) (Martin 2013).

Surveys were conducted in late summer to early autumn in the native range, from Aug to Oct 2018. These mo were chosen for the surveys to ensure that the insect populations had most of the growing season to establish. This also was the best time for surveys to occur logistically. Sites were identified by contacting personnel at state universities and state wildlife and fisheries departments via email and by telephone. This was necessary as herbarium and survey records did not provide GPS coordinates of the sites, and populations of *N. mexicana* fluctuate with changes in water levels due to rainfall, flooding, and hurricanes. Sites were accessed by motorboat or airboat, and the leaves, stems, roots, and flowers of *N. mexicana* were inspected for damage. All life stages of insects were collected using an aspirator, Berlese funnel extractions, and manually by inspection under microscope in the laboratory. Specimens were frozen or preserved in 70 to 95% isopropyl or ethyl alcohol for later identification. An attempt was made to rear live collected insects under laboratory conditions. Whole potted plants were placed in 68 L black mesocosm tubs in a greenhouse at the University of Florida, Fort Lauderdale Research and Education Center, Davie, Florida, USA, and USDA facility in Davie, Florida, USA, and insects collected in the field were placed on the plants and left to feed while observations were made. Temperatures in the greenhouse are estimated to fluctuate around 27 °C. Leaf samples were taken from each site and dried in zipper top plastic bags with silica gel or Drierite for DNA analysis. Eighteen sites were surveyed at 17 different water bodies (Fig. 1).

Where possible, all parts of *N. mexicana* plants were searched for all life stages of insect natural enemies, including root tubers, stems, leaves, and flowers. However, this was not always possible because the roots and tubers could not be pulled successfully from the sediment in deeper areas. Individual based rarefaction species accumulation curves were generated using the Chao 2, incidence coverage estimator, and MMRuns estimators in EstimateS version 8.0 (Colwell 2006) because these estimators are effective at estimating true species diversity when the abundances of species are unknown. Incidental insect visitors to *N. mexicana* (that is, those that did not feed on the plant, as determined by observation and literature searches) were excluded from the analyses. Species were prioritized based on extent of feeding damage and mode of damage, incidence, field host range, and climate matching (Spafford & Briese 2003; Paterson 2010). Prioritization based on genotype matching was limited because genetic work has been conducted only on material from 2 sites (Naidu 2018).

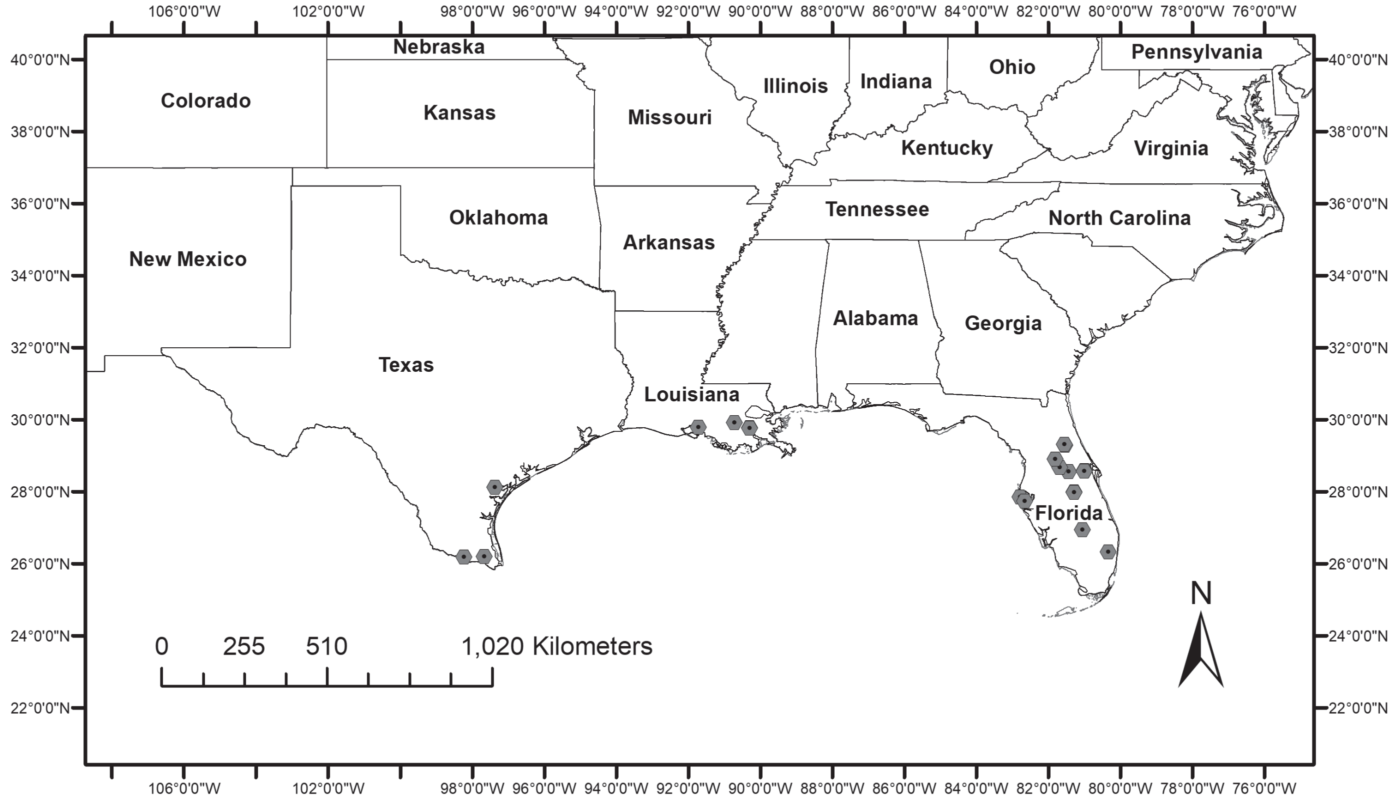

Fig. 1. Sites surveyed for natural enemies of *Nymphaea mexicana* in the southeastern USA during Aug to Oct 2018. Map created using ArcMap (Environmental Systems Research Institute 2014).

HOST SPECIFICITY PILOT STUDY

As a preliminary assessment of the host specificity of the most promising agent, *Bagous americanus* LeConte (Coleoptera: Curculionidae), 5 outside concrete crypt tanks (2.4 m long × 0.9 m high × 0.9 m wide) with lids were prepared by adding 1 plant each of *N. mexicana*, *Nymphaea odorata* Ait., *Nuphar lutea* (L.) Sm. (all Nymphaeaceae), *Sagittaria latifolia* Willd. (Alismataceae), *Nymphoides indica* (L.) Kuntze (Menyanthaceae), and *Cabomba caroliniana* A. Gray (Cabombaceae) to each tank. The tanks were situated at the University of Florida, Fort Lauderdale Research and Education Center, and USDA facility in Davie, Florida, USA. Eight *B. americanus* adults were added to the tanks 1 d after the plants to allow the plants to acclimatize before the insects were introduced. All plants were inspected and photographed, and observations and measurements of the mines were taken every 2 to 3 d over a period of 20 d. An additional 68 L black mesocosm tub with 2 *N. mexicana* plants was kept in a greenhouse and covered with netting. Sixteen *B. americanus* weevils were added to this mesocosm, and observations and measurements of mines were made over the same time period. The mean (± SE) length of the mines was measured from photographs using Image J (Schneider et al. 2012). At the end of the experiments, all plants were thoroughly inspected, and stems were dissected to detect the presence of *B. americanus* larvae and pupae.

Results

MAXENT MODELLING

MaxEnt modelling indicated that the regions most climatically similar to the invaded range of *N. mexicana* in South Africa were the southeastern USA, from the southern half of Georgia, into Florida, and along the lower southern parts of Alabama, Mississippi, Louisiana, and southeastern Texas. Climatically suitable areas also occur in the eastern and western regions of central Mexico (Fig. 2).

SURVEYS

Sampling Effort

Most of the species present on *N. mexicana* were sampled during these surveys, as the species accumulation curve almost approached the asymptote (Fig. 3). The incidence coverage estimator mean was slightly higher than the S(est) curve and estimated that 1 or 2 species were missed during the surveys, whereas the MMRuns mean had a slightly steeper curve and also estimated that further surveys may reveal the presence of 1 or 2 more species. Similarly, the Chao 2 mean was higher than the S(est) curve, but reached the same point as the curve ends, whereas the Chao 2 upper 95% confidence interval suggests that up to 5 species may be unrecorded on *N. mexicana*. Hence, overall 2 to 5 species could have been missed during the surveys.

Survey Outcomes

The surveys took place in the late summer to early autumn months in the USA. Many of the sites surveyed had healthy populations of *N. mexicana* isolated from other *Nymphaea* species, although a few sites, namely Big Lake in Welder Wildlife Foundation Management Area, San Patricio County, Sinton, Texas, USA, and Lake Okeechobee in Glades County, Lake Kissimmee in Osceola County, and the Everglades in Broward County, Florida, USA, were near to or mixed with populations of *Nymphaea elegans* Hook. (Nymphaeaceae) (Big Lake) or *N. odorata* (Table 1). The sites surveyed consisted of large natural lakes and wet-

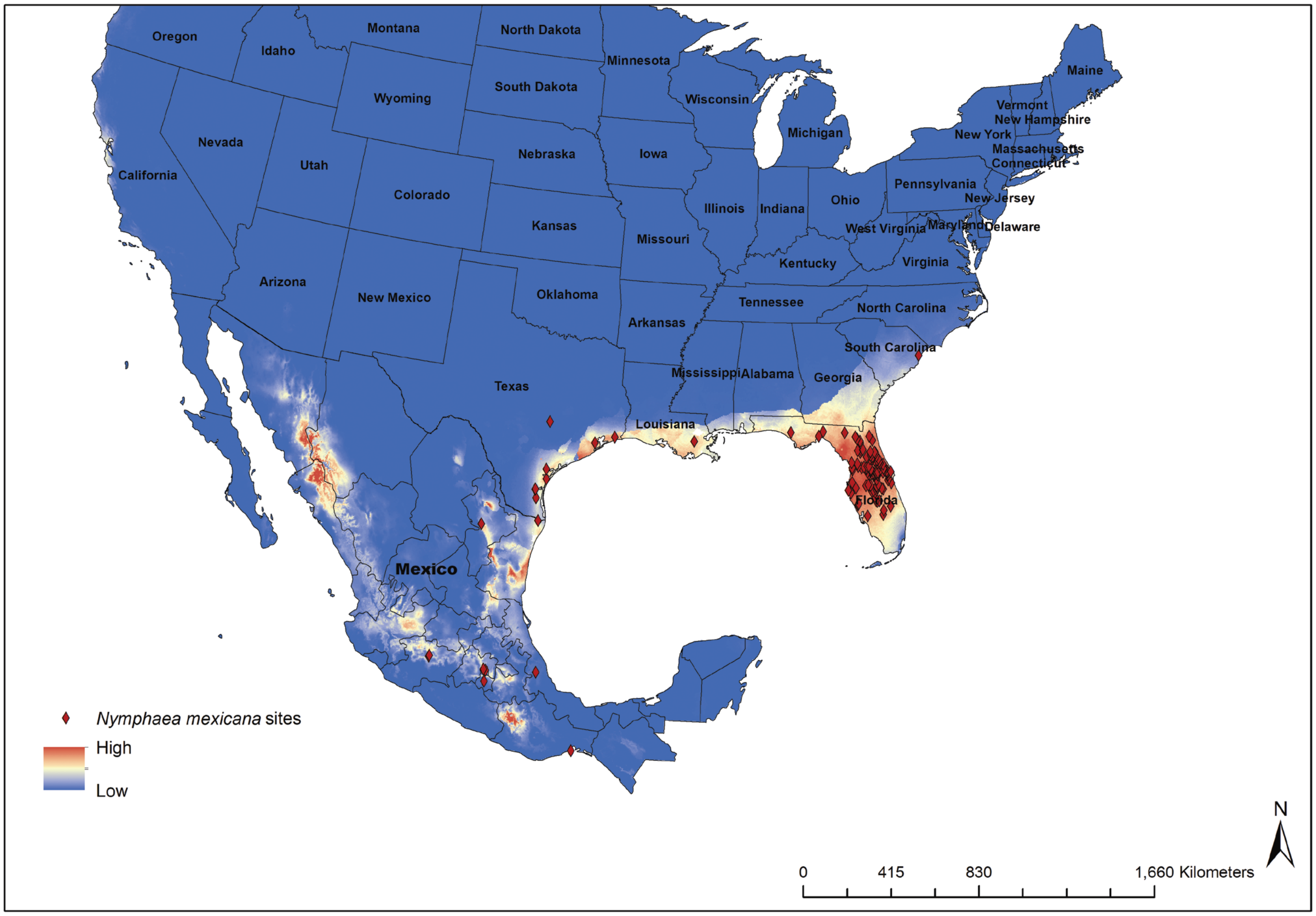

Fig. 2. Climatic similarity of the native and introduced distributions of *Nymphaea mexicana* in South Africa, modelled using MaxEnt (Phillips et al. 2004, 2006). Red colors indicate regions that are climatically similar to South Africa, and lighter yellow and blue coloring indicates regions of lower similarity. Purple icons indicate the native range sites considered in the analysis.

lands accessible only by airboat, smaller lakes accessible by motorboat or from the bank, and a few canals and artificial ponds. Almost all *N. mexicana* populations were exposed to full sunlight, and the water depth at the sites ranged from knee deep to about 1.8 m. Populations of *N. mexicana* ranged from patches of 1 or 2 plants to stands of hundreds of large, healthy flowering plants covering areas of over 50 m^2. Numerous aquatic insects were encountered during the surveys, and several caused feeding damage on *N. mexicana* (Table 2). At most sites, there was considerable damage associated with the presence of generalist lepidopteran species, such as *Elophila* spp. and *Parapoynx* spp., as determined by literature searches. Generalist herbivore insects were found during the surveys, including *Notiphila latigena* Mathis (Diptera: Ephydridae) (identified by Richard Zack, Washington State University, Pullman, Washington, USA); *Draeculacephala* sp. (Hemiptera: Cicadellidae); a black planthopper, suspected to be *Megamelus davisi* Van Duzee (Hemiptera: Delphacidae); and *Donacia cincticornis* Newman and *Donacia hypoleuca* Lacordaire (both Coleoptera: Chrysomelidae) (identified by Chris Carlton and Victoria Bayless, Louisiana State University, Baton Rouge, Louisiana, USA). Chironomid larvae that were collected at several sites created 'trenches' in the leaf surface and caused considerable damage, but also were recorded from Berlese funnel extractions on *Nymphaea pubescens* Willd. (Nymphaeaceae) (synonym *Nymphaea rubra* Roxb. ex Andrews), and *N. odorata*. However, these larvae were not identified to species level because larvae are difficult to distinguish. Coccinellids believed to be feeding on the aphids present at a site in Texas, a small number of lampyrids, luminous green dipterans, Sciaridae, Gerridae, and a few parasitoids (Ichneumonidae), all were recorded at 1 or more sites.

Megamelus toddi Beamer (Hemiptera: Delphacidae) (identified by Andy Boring and Susan Halbert, Florida Department of Agriculture and Consumer Services, Gainesville, Florida, USA) (Fig. 4) was found at 39% of the sites (Table 1). In Louisiana, *M. toddi* populations of several thousand were found at the Cote Blanche Crossing site (29.7774°N, 91.7155°W), and this species also was relatively abundant at Lake Boeuf, Lafourche Parish, Louisiana, USA.

Lysathia ludoviciana Fall (Coleoptera: Chrysomelidae) (identified by Paul Skelley, Florida Department of Agriculture and Consumer Services, Gainesville, Florida, USA, and Brian Bahder, University of Florida, Gainesville, Florida, USA) also was found feeding on *N. mexicana*, though only at 2 of the sites. Only adults were found, and these were taken back to the laboratory where they laid eggs, and the larvae fed on *N. mexicana* leaves. However, after only 1 or 2 d, the larvae no longer were seen on the leaves. It is possible that the larvae moved away or died, but no adults emerged in the remaining 2 wk before the experiments were terminated. A single adult survived on *N. mexicana* for several wk in the laboratory. However, *L. ludoviciana* is known to complete its life cycle on *Myriophyllum aquaticum* (Velloso) Verde (Haloragaceae) even though this is an introduced plant in the beetle's native

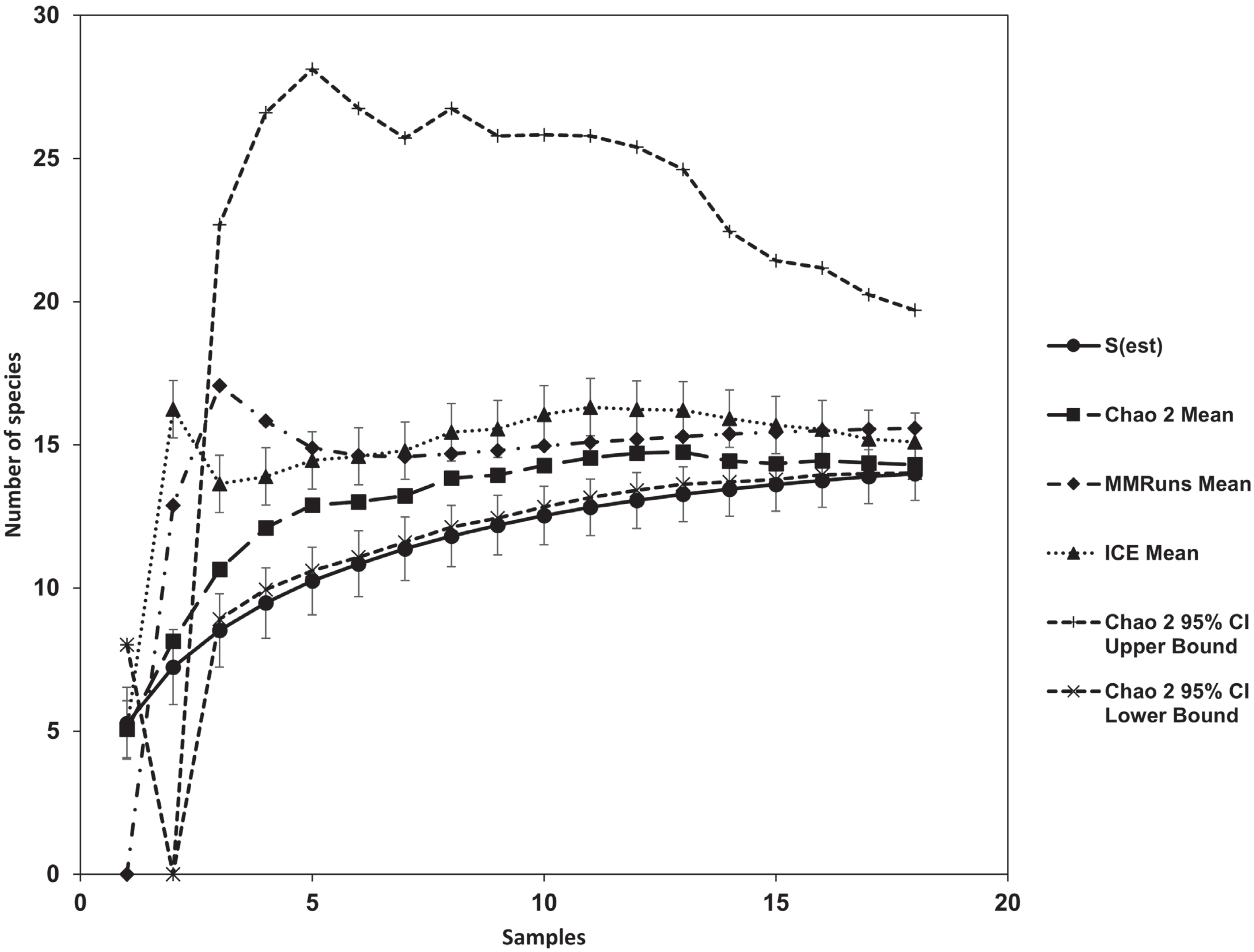

Fig. 3. Species accumulation curve showing incidence coverage estimator (ICE) mean, Chao 2 mean, and the Chao 2 95% upper and lower confidence intervals, and MMRuns mean indicators for species richness (error bars indicate standard deviations). Chao 2 mean estimates the true species diversity based on incidence data; ICE mean is the incidence coverage estimator, and the MMRuns (Michaelis-Menton) mean calculates the mean score after 100 randomizations (Colwell 2006; Gotelli & Colwell 2011).

range (Habeck & Wilkerson 1980), as well as *Ludwigia* sp. (Onagraceae) (McGregor et al. 1996), so it is unlikely to be suitable as a biological control agent for *N. mexicana*.

Bagous americanus (Robert Anderson, Canadian Museum of Nature, Ottawa, Ontario, Canada) was found completing its life cycle on *N. mexicana* at all 4 sites surveyed in Louisiana, causing considerable damage to the plants (Fig. 4). The adults were found in curled up, browning leaf edges, but the larvae and pupae were much more commonly found by dissecting the petioles of leaves showing characteristic mining patterns towards the centre of the leaf (Fig. 4). Adults were collected and taken back to the laboratory, where they successfully completed a life cycle.

Although *N. odorata* is a known host for *B. americanus* (McGaha 1954; Cronin et al. 1998), there are no records of this weevil species occurring on *N. mexicana* or any other species. Additionally, there were no *N. odorata* plants at any of the sites where *B. americanus* was recorded on *N. mexicana* during the surveys. *Bagous americanus* was not found during the initial surveys in Florida, but 1 site (Lake Seminole, Pinellas County, Florida, USA) was resurveyed opportunistically, and plant material from 2 other sites (Lawne Lake in Orange County, Florida, USA, and Lake Okeechobee) were mailed by Florida Fish and Wildlife Conservation Commission to be searched for the weevils toward the end of the surveying trip. Plant material from 1 of these sites (Lake Okeechobee) contained weevil larvae suspected to be *B. americanus*, but confirmation is needed. This site had a large population of *N. odorata* growing with *N. mexicana*, so it is possible that these plants could be hybrids.

HOST SPECIFICITY PILOT STUDIES

The first signs of mining by *B. americanus* during preliminary host-specificity trials conducted in outside tanks were noticed on 10 Oct 2018, 4 d after the weevils had been added. By 15 Oct 2018, many of the mines reached the petiole. Oviposition and larval mining occurred only on *N. mexicana* and *N. odorata*. Mines were recorded on 15 of approximately 86 *N. mexicana* leaves (17.44%), and 3 of approximately 11 *N. odorata* leaves (27.27%) across the 5 tanks.

In the greenhouse mesocosm, mining by *B. americanus* larvae was noticed on 10 Oct 2018, 4 d after the weevils had been added. By 15 Oct 2018, most mines had reached the petioles. Eleven leaves had mines, and some had 3 or 4 mines on 1 leaf. The mean mine length, measured from the outside tanks as well as the greenhouse mesocosm

Table 1. Sites surveyed for natural enemies of *Nymphaea mexicana* in the southeastern USA, and other plant species present at the sites in 2018. In the table, common plant names are listed.[a]

Site	State	Latitude, Longitude	Plants present
Cote Blanche Crossing	Louisiana	29.7774°N, 91.7155°W	Hornwort, water hyacinth, sawgrass, other grasses
Lake Boeuf	Louisiana	29.9111°N, 90.7117°W	Water hyacinth, hydrilla, frog's bit, duckweed, hornwort, salvinia, smartweed, american water lotus, alligatorweed
Salvador site 1	Louisiana	29.7657°N, 90.2930°W	Hornwort, hydrilla, water hyacinth, grasses, salvinia, american water lotus
Salvador site 2	Louisiana	29.7623°N, 90.2917°W	Hornwort, hydrilla, american water lotus, water hyacinth, fanwort, pennywort, common water nymph
Harlingen roadside canal	Texas	26.1903°N, 97.6636°W	Water hyacinth, some grasses
Big lake, Welder Wildlife Foundation Management Area	Texas	28.1216°N, 97.3650°W	Tropical royalblue waterlily, american water lotus, cattail, *Naja* sp., bladderwort, sesbania, dollarweed, cattail, smartweed
Quinta urban park	Texas	26.1767°N, 98.2298°W	Cattail, various bank plants
Lawne Lake	Florida	28.5579°N, 81.4381°W	Spatterdock, water hyacinth, hydrilla, pickerelweed, spikerush
Lake Apopka	Florida	28.6722°N, 81.6748°W	Sagittaria, pickerelweed, hydrilla
Lake George	Florida	29.2828°N, 81.5408°W	Alligatorweed eelgrass, hornwort, hydrilla, water lettuce, salvinia, cattail
Lake Kissimmee	Florida	27.9792°N, 81.2743°W	Frog's bit, hydrilla, salvinia, ludwigia, hornwort, spatterdock, water lettuce
Lake Okeechobee	Florida	26.9329°N, 81.0503°W	White waterlily, pickerelweed, bladderwort, hornwort, smartweed, cattail
Lake Seminole	Florida	27.8414°N, 82.7740°W	Pickerelweed, grasses, eelgrass, sagittaria, spikerush, hydrilla
Lake Maggiore	Florida	27.7373°N, 82.6475°W	Hydrilla, cattail
Pine Island lodge	Florida	29.3119°N, 81.5458°W	Hornwort, alligatorweed, cattail
Emeralda marsh near Lake Griffin	Florida	28.9039°N, 81.8087°W	Hydrilla, sagittaria, alligatorweed
Orlando wetlands park	Florida	28.5824°N, 81.0022°W	Hornwort, pickerelweed, pennywort, common water nymph, hydrilla, duckweed, ludwigia
Everglades	Florida	26.3205°N, 80.3300°W	White waterlily, dollarweed, common water nymph, spikerush, pickerelweed

[a]The species names and families can be found in the following descriptions, which are listed alphabetically by common name: Alligatorweed, *Alternanthera philoxeroides* (Mart.) Griseb (Amaranthaceae); american water lotus, *Nelumbo lutea* Willd. (Nelumbonaceae); bladderwort, *Utricularia* L. (Lentibulariaceae); cattail, *Typha* spp. L. (Typhaceae); common water nymph, *Naja guatalupensis* (Spreng.) Magnus (Hydrocharitaceae); hornwort, *Ceratophyllum demersum* L. (Ceratophyllaceae); dollarweed, *Hydrocotyle umbellata* L. (Araliaceae); duckweed, *Lemna* spp. L. (Araceae); eelgrass, *Vallisneria* L. (Hydrocharitaceae); fanwort, *Cabomba caroliniana* A. Gray (Cabombaceae); floating primrose willow, *Ludwigia peploides* (Kunth) P.H. Raven; frog's bit, *Limnobium spongia* (Bosc) L.C. Rich. ex Steud. (Hydrocharitaceae); hydrilla, *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae); ludwigia, *Ludwigia* spp. L. (Onagraceae); pennywort, *Hydrocotyle* spp. L. (Araliaceae); pickerelweed, *Pontederia cordata* L. (Pontederiaceae); sagittaria, *Sagittaria* spp. L. (Alismataceae); salvinia, *Salvinia molesta* D. Mitch. or *Salvinia minima* Baker (Salviniaceae); sawgrass, *Cladium* P. Browne (Cyperaceae); sesbania, *Sesbania* spp. Scopoli (Leguminosae); smartweed, *Polygonum* L. (Polygonaceae); spatterdock, *Nuphar advena* (Aiton) W.T. Aiton (Nymphaeaceae); spikerush, *Eleocharis* spp. (L.) Roem. & Schult. (Cyperaceae); tropical royalblue waterlily, *Nymphaea elegans* Hook (Nymphaeceae); water hyacinth, *Pontederia crassipes* Mart. [□ *Eichhornia crassipes* (Mart.) Solms] (Pontederiaceae); waterlettuce, *Pistia stratiotes* L. (Araceae); white waterlily *Nymphaea odorata* Aiton (Nymphaeaceae).

Table 2. Summary of species found on *Nymphaea mexicana* during surveys in the southeastern USA during 2018. Species identifications marked by an asterisk were not confirmed by a taxonomist. Incidence values indicate the number of sites, expressed as a percentage, at which each species was present. Levels of feeding damage were estimated subjectively and comparatively, and potential for use in biological control is based on feeding damage and incidence, as well as likely host specificity as determined from information in the literature and observations of host range during the surveys.

Family	Species (author)	Life stage	Feeding	Level of feeding damage	Incidence (%)	Potential for use in biological control
Curculionidae	*Bagous americanus* (LeConte)	Adult	Leaf chewer	Low	17	–
		Larvae	Mines leaves and petiole	High	17	High
Delphacidae	*Megamelus toddi* (Beamer)	Adults and nymphs	Sap sucker	Low to medium	39	Medium to high
	Megamelus sp. (*davisi?*) (Van Duzee)	Adults and nymphs	Sap sucker	Low	11	Low
Chrysomelidae	*Donacia* spp.	Larvae	Root feeder	Uncertain	–	–
	(F.)	Adult	Leaf feeder	Medium	67	Medium
	Donacia hypoleuca (Lacordaire)		As above for *Donacia* spp.	Uncertain	Uncertain	Medium
	Donacia cincticornis (Newman)		As above for *Donacia* spp.	Uncertain	Uncertain	Low
	Lysathia ludoviciana (Fall)	Larvae	Not observed in field, but leaf chewing in laboratory	–	–	–
		Adult	Leaf chewing	Uncertain	11	Low
Crambidae	*Parapoynx* spp.* (Hübner)	Larvae	Leaf chewing, bore into petiole	High	83	Low
	Elophila/Synclita spp.* (Walker)	Larvae	Leaf chewing	High	89	Low
Ephydridae	*Notiphila latigena* (Mathis)	Pupae	On roots	Uncertain	100	Low
Diptera	Unknown		Uncertain. Possibly *Hydrellia* fly associated with *Hydrilla verticillata*	Uncertain	17	Probably low
Arctiidae	*Spilosoma virginica* * (F.)	Larvae	Leaf chewing	High	6	Low
Cicadellidae	Unknown (suspected *Draeculacephala* sp. (Ball))	Adults and nymphs	Sap sucker	Uncertain	39	Uncertain
Aphididae	Unknown	Adults and nymphs	Sap sucker	Uncertain	6	Low
Chironomidae	Unknown	Larvae	Leaf grazing (produce trenches)	High	28	Unclear

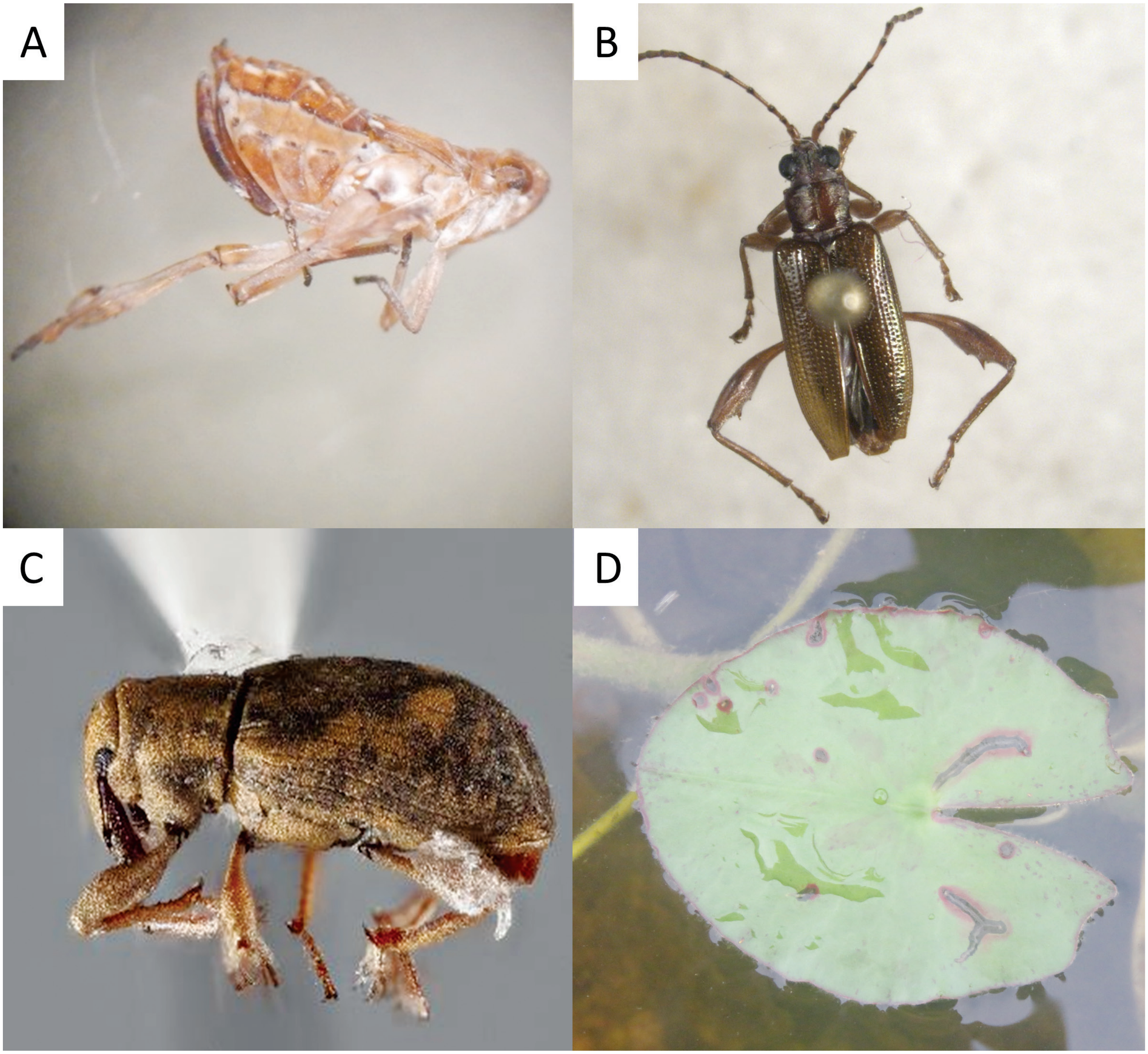

Fig. 4. Natural enemies and feeding damage on *Nymphaea mexicana*. (A) *Megamelus toddi* Beamer (Hemiptera: Delphacidae); (B) *Donacia* sp. F. (Coleoptera: Chrysomelidae); (C) *Bagous americanus* LeConte (Coleoptera: Curculionidae); (D) mines produced by *Bagous americanus* larvae.

once mines had reached the petiole, was 2.7 ± 0.4 cm (SE; *n* = 12). The mean boring distance in the petiole was 0.8 ± 0.1 cm (SE; *n* = 5). At the end of the experiment, the petioles were dissected from the outside tanks and the mesocosm, and pupae were found in 2 *N. mexicana* petioles, 1 *N. mexicana* petiole had a newly formed adult, and a larva was found in a *N. odorata* petiole. Other dissected petioles showed signs of mining and boring but no larvae or pupae, and some showed evidence of emergence holes.

Discussion

The species richness of natural enemies found on *N. mexicana* is lower than that of *N. odorata*, which hosts various Trichoptera, Lepidoptera, and Coleoptera (Harms et al. 2011). However, the insect taxa found on *N. odorata* have been investigated more extensively. Furthermore, many of the species found during these surveys were not identified to species. The species accumulation curve indicators suggest that about 2 to 5 species were likely to have been missed during the surveys. These indicators are useful to estimate species richness, and the Chao 2 and incidence coverage estimators have proven to perform well and may present higher accuracy than asymptotic estimators, such as the Michaelis-Menton and Bootstrap estimators (Hortal et al. 2006). However, only 17 sites were sampled during the surveys, and it is recommended that rarefaction should be based on at least 20 samples or more (Gotelli & Colwell 2011).

Sampling effort, sampling periods, and seasonal variability may have affected the species abundance and diversity. These surveys were conducted in the late summer to early autumn mo, so other species could be present at different times of the yr. Furthermore, many of the natural enemies observed on *N. mexicana* were identified only to family, while some individual species identifications, especially the chironomids, *Parapoynx* Hübner (Lepidoptera: Crambidae) and *Elophila* Walker (Lepidoptera: Crambidae) species, were not confirmed. Hence, it is possible that there could be additional species hosted by *N. mexicana* that were not identified during these surveys, and it may be useful to conduct additional surveys at additional sites. Most of the species found on *N. mexicana* during the surveys, including unidentified Chironomidae and Lepidoptera, as well as *N. latigena*, *M. davisi*, and *Donacia* spp. either are known to be generalists based on literature searches or are not associated with plant damage.

Megamelus toddi was found in abundance at all the sites surveyed in Louisiana, as well as a few sites accompanied by what is thought to be *M. davisi* in Florida. *Nuphar advena* Ait. (Nymphaeaceae) and *N. odorata* are known hosts of *M. davisi* (McGaha 1952; Harms & Grodowitz 2009), so it is unlikely to be host specific to *N. mexicana*, and its abundance was not sufficient on the plants to warrant collection. Furthermore, *N. advena* and *N. odorata* often were present at the sites where *M. davisi* was found. The record acquired from these surveys was the first record of *M. toddi* being hosted by waterlilies and also was a new county record (S. Halbert, personal communication). *Megamelus toddi* was particularly abundant at a few of the sites surveyed, though the level of damage was not very high at the time of sampling. However, it is possible that effects on the plant would be seen only later. Furthermore, *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) has been released in South Africa to control water hyacinth, *Pontederia crassipes* Mart. [≡ *Eichhornia crassipes* (Mart.) Solms] (Pontederiaceae) (Pellegrini et al. 2018), and is well established and damaging in cooler regions of the country where other biological control agents have struggled to establish (Hill & Coetzee 2017). Therefore, due to the lack of information on its biology and host range, and the known success of *M. scutellaris*, *M. toddi* has potential as a biological control agent of *N. mexicana*.

Although *N. odorata* is a known host of *B. americanus*, a feeding selectivity study conducted by Cronin et al. (1998) demonstrated that *B. americanus* preferred to feed on *N. odorata* when offered a choice of *Nuphar variegata* Engelman and *Nuphar pumila* (Pers.) Fernald (Nymphaeaceae), *Pontederia cordata* L. *Potamogeton amplifolius* Tuckerman (both Pontederiaceae), *Typha latifolia* L. (Typhaceae), and *Calla palustris* L. (Araceae). Results from the host specificity pilot tests conducted in this study similarly suggest that the species is specific at least to the genus *Nymphaea*. In the literature, there are no published records of other *Nymphaea* species aside from *Nymphaea tuberosa* Paine (Nymphaeaceae) as hosts for *B. americanus* (McGaha 1954; Harms & Grodowitz 2009). However, it has since been determined that *N. tuberosa* does not possess enough genetic difference from *N. odorata* to be classified as a separate species and should, therefore, be demoted to subspecies level (*Nymphaea odorata* subsp. *tuberosa* (Paine) Wiersma & Hellq) (Woods et al. 2005).

It is necessary to conduct further studies to quantify the damage exerted by *B. americanus* and *M. toddi* on *N. mexicana*, and to evaluate plant responses attributed to such feeding damage. However, of the 15 species found during these surveys, *B. americanus* and *M. toddi* have the greatest potential to act as biological control agents of *N. mexicana* based on observations of field densities, climatic adaptations, field host associations in the native range, and the success of similar congeners as biological control agents on other plants. Hence, we suggest that *B. americanus* and *M. toddi* should be prioritized for importation, and their host specificity studied under quarantine conditions at Rhodes University, Grahamstown, Eastern Cape, South Africa.

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