

THE AFRICAN BURROWING MAYFLY, *POVILLA ADUSTA*
(EPHEMEROPTERA: POLYMITARCYIDAE), DAMAGES *HYDRILLA*
VERTICILLATA (ALISMATALES: HYDROCHARITACEAE)
IN LAKE TANGANYIKA

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ABSTRACT

The submersed aquatic plant, *Hydrilla verticillata* (L.f.) Royle (hydrilla) is a serious invasive weed in the southern USA, but in Central African lakes, it is not considered a problem. Previous surveys in Lake Tanganyika identified 2 species of chironomid midges in the genus *Polypedilum* Kieffer that putatively caused tip-mining damage to hydrilla. We returned to Lake Tanganyika to further investigate the midges and to explore for other natural enemies of hydrilla. High proportions of stems collected at 2 locations south of Bujumbura were found to have bored apical meristems. No insects were observed in the burrows, but large numbers of nymphs of the African burrowing mayfly, *Povilla adusta* Navas, were dislodged when hydrilla stems were shaken under water. Because *P. adusta* is known to burrow in living aquatic plants, wood and several inorganic substrates, we hypothesized that *P. adusta* nymphs caused the boring damage to hydrilla. To test this hypothesis, undamaged hydrilla was exposed to *P. adusta* nymphs in a series of laboratory experiments. In all experiments combined, 4% of hydrilla stems exposed to *P. adusta* were damaged, whereas no stems in containers without *P. adusta* were damaged. Damage was characterized as lateral or vertical boring in apical meristems and, less frequently, in stems. To quantify the level of damage in the field, stems were collected from 2 locations on 6 occasions. The proportions of stems with bored apical meristems ranged from 5-52% and 13-57% of stems were missing apical meristems. Although *Povilla adusta* caused substantial damage to hydrilla in Lake Tanganyika, it should not be considered for biological control because it is not a herbivore, and is known to burrow in a wide variety of substrates. The *Polypedilum* spp. midges earlier reported to bore into hydrilla apical meristems were not abundant in the field and not found associated with boring damage. The damage earlier attributed to the midges was likely due mostly or entirely to *P. adusta*. These midges should not be pursued further as candidate biological control agents of hydrilla.

Key Words: *Polypedilum*, tunneling, aquatic weed, biological control, Burundi, Africa

RESUMEN

La planta acuática sumergida, *Hydrilla verticillata* (L.f.) Royle (hydrilla), es una seria maleza invasiva en el sur de los Estados Unidos, pero en lagos de África Central, esta no es considerada un problema. Muestreos previos en el Lago Tanganyika identificaron dos especies de moscas chironomidas en el género *Polypedilum* Kieffer que posiblemente ocasionaron un daño de minado en los brotes de hydrilla. Nosotros regresamos al Lago Tanganyika para investigar más estas moscas y para explorar por otros enemigos naturales de hydrilla. Una alta proporción de tallos colectados en dos localidades al sur de Bujumbura tuvieron los meristemas apicales barrenados. No se observó insectos en las galerías formadas, pero un número grande de ninfas de la Mosca de Mayo Africana barrenadora, *Povilla adusta* Navas, fueron obtenidos cuando se sacudió los tallos de hydrilla bajo agua. Debido a que *P. adusta* es conocida por barrenar en plantas acuáticas vivas, madera y algunos substratos inorgánicos, se hipotetizó que las ninfas de *P. adusta* causaron el barrenado en hydrilla. Para probar esta hipótesis, se expusieron plantas de hydrilla sin daño a las ninfas de *P. adusta* en una serie de experimentos de laboratorio. En todos los experimentos, 4% de los tallos de hydrilla expuestos a *P. adusta* fueron dañados, mientras que ningún tallo en los contenedores sin *P. adusta* fueron dañados. El daño fue caracterizado como barrenado lateral o vertical en los

meristemas apicales, y con menos frecuencia, en los tallos. Para cuantificar el nivel de daño en el campo, se colectó tallos de dos localidades en seis ocasiones. La proporción de tallos con meristemas apicales barrenados varió entre 5-52% y 13-57% de los tallos no tuvieron meristemas apicales. A pesar de que *Povila adusta* causó daño substancial a hydrilla en el Lago Tanganyika, este insecto no debería ser considerado para control biológico porque no es un herbívoro, y además es conocido por barrenar en una variedad de substratos. Las moscas *Polypedilum* spp. reportadas anteriormente como barrenadoras en los meristemas apicales de hydrilla no fueron abundantes en el campo y no fueron asociadas con un daño de barrenado. El daño anterior atribuido a las moscas fue probablemente en su mayor parte o en su totalidad debido a *P. adusta*. Estas moscas chironomidas no deberían ser consideradas como candidatas para control biológico de hydrilla.

Hydrilla verticillata (L.f.) Royle (Hydrocharitaceae) (hereafter, hydrilla) is a rooted, submersed aquatic plant that first invaded water bodies in Florida in the 1950s (Schmitz et al. 1991), and is now considered to be one the most serious freshwater aquatic weeds in the southeastern United States (Balciunas et al. 2002). Hydrilla interferes with water flow, recreational activities, and displaces native aquatic plant communities (Langeland 1996). The native range of hydrilla includes much of Asia and northern Australia, and there are also disjunct populations in Europe and East Africa (Cook & Lüönd 1982). The first African record of hydrilla was in 1862 in the Nile River near its source in Lake Victoria (Speke 1864). This early report, coupled with hydrilla's lack of weediness (Pemberton 1980; Verklieij et al. 1983; Gidudu et al. 2011), suggests that it may be native to Africa.

Because of hydrilla's economic importance and status as an introduced weed, it has been the subject of biological control research since the early 1970s. Exploration for natural enemies has been conducted in a number of Asian countries and in East Africa (for reviews see Buckingham 1994; Balciunas et al. 2002). Surveys by Pemberton (1980) and Markham (1985) in Lake Tanganyika found evidence of damage to hydrilla, and both studies concluded that the damage was caused by chironomid midges in the genus *Polypedilum* Kieffer. Damage was characterized as boring in the apical meristems and the upper part of the stems. The insects were collected and shipped to Florida in 1990 and identified as *Polypedilum wittei* Freeman and *Polypedilum dewulfi* Goetghebuer by John Epler (Buckingham 1994). Efforts to establish laboratory colonies of these midges in Florida failed (Buckingham 1994).

In addition to the *Polypedilum* spp., Pemberton (1980) observed 3 instances of the mayfly, *Povilla adusta* Navas (Polymitarcyidae), boring in basal portions of hydrilla stems. This mayfly is the only member of its genus to occur in Africa (Corbet 1974; Hubbard 1984), and its biology has been investigated by several authors. Nymphs use well-developed mandibles to burrow into a number of different substrates, and then line the burrows with a silk-like substance secreted from the anus (Hartland-Rowe 1958). Early instars are filter feeders,

creating a current within the burrow by rapidly moving their gills. Later instars graze on periphyton (Hare & Olisedu 1987). *Povilla adusta* has been reported to burrow in wood (submersed trees and boats) (Hartland-Rowe 1958; McLachlan 1970; Petr 1970), fresh water sponges (Arndt 1938, cited in Hartland-Rowe 1958), holes in laterite (Hartland-Rowe 1953), shell fragments, gravel, sandstone rocks (Bidwell 1979), and sandy-clay sediments hardened by previous exposure to air (Hare & Olisedu 1987). Mature nymphs have been collected from burrows chewed into thick rubber gaskets used to seal a water intake pipe in Lake Victoria, Kenya (Copeland, unpublished data). They also are known to bore into the stems and roots of living aquatic plants including *Pistia stratiotes* L. (Dejoux 1969), *Persicaria senegalensis* (Meisn.) Soják (as *Polygonum senegalense*), *Phragmites* sp., *Cyperus papyrus* L. (Petr 1973) and *Cyperus denudatus* L. f. (Hartland-Rowe 1958). According to Petr (1973), they do not feed on the substances in which they burrow.

The recent discovery of herbicide resistant populations of hydrilla in Florida (Michel et al. 2004) stimulated renewed interest in foreign exploration for biological control agents (Overholt & Wheeler 2006). As part of this effort, surveys were conducted from 2006-2010 in Lake Tanganyika in Burundi and several lakes in Uganda. A major objective of the work in Africa was to locate and initiate laboratory colonies of one or both of the aforementioned *Polypedilum* species so that host range and other biological studies could be conducted. For the greater part of our study, insect damage to hydrilla was not observed either in Burundi or in 4 Ugandan lakes (Copeland, unpublished data). However, in Feb 2010, high proportions of the apical meristems of hydrilla collected at 2 locations on the Burundi shore of Lake Tanganyika were found to be damaged. The damage was characterized by lateral and, less often, vertical tunneling through the growing tip (Figs. 1 and 2), but no organisms were observed in the tunnels. However, when stems were shaken under filtered water collected from the same sites, many *P. adusta* nymphs were dislodged. Based on the presence of *P. adusta*, we hypothesized that the mayfly was responsible for the boring damage



Fig. 1. Bored apical meristem of hydrilla collected at Rumonge. Note that in an unopened bud the holes overlap to form a single burrow.

to hydrilla. The objectives of the present study were to determine through controlled laboratory experiments whether *P. adusta* was responsible for the boring damage to hydrilla tips, and to quantify the level of damage at selected locations in Lake Tanganyika.

MATERIALS AND METHODS

Laboratory Experiments

Hydrilla used in laboratory experiments was harvested from either a Lake Tanganyika site (experiments 1-4) near Bujumbura where bored meristems were never observed during repeated sampling from 2006-2010 (Copeland, unpublished) or from a laboratory culture growing in a

large outdoor aquarium enclosed in a insect-proof wire-mesh cage (experiments 5-7). To establish the laboratory culture, stems were hand pulled from wild plants growing at the Bujumbura site and transplanted into the outdoor aquarium. The growing medium in the aquarium was heat-sterilized mud from the Bujumbura site. Plants were grown for 1 month prior to initiation of experiments.

In each experiment, 12 distal stem portions (hereafter referred to as 'sprigs'), each about 12 cm long and with an intact growing tip, were cut and transplanted into plastic containers (16 cm × 17 cm × 24 cm). In experiments 1-4 heat-sterilized mud was used as the substrate into which hydrilla was transplanted, whereas in experiments 5-7, washed construction sand was used to decrease turbidity in containers. On the d that each experiment was initiated, hydrilla was collected from Rumonge—one of the sites where bored apical meristems had been found—and transported to the laboratory. Plants were shaken under filtered water collected at the same site and *P. adusta* nymphs were captured as they swam free of their disturbed habitat.

In experiments 1-6, 2 containers were randomly selected as controls and received no nymphs whereas the remaining 6 or 10 containers each received the same number of nymphs. The number of nymphs added varied from 7-31, depending on the abundance of *P. adusta* at the time hydrilla was sampled. In experiment 7, 9 treatment containers received *P. adusta*, and 5 containers were controls. Details of the experimental conditions for each trial are provided in Table 1. Containers were left undisturbed for 1 wk, after which each sprig was removed and inspected under a dissecting microscope for signs of damage. The number of sprigs with damage was compared between containers with and without *P. adusta* with Fisher's Exact Test.

Field Sampling

Two collections of hydrilla were made from Magara (S3.720°, E29.307°) and 4 collections from Rumonge (S3.979°, E29.435°) in the littoral zone of Lake Tanganyika, approximately 5-25 m offshore. Plant material was collected either by throwing a grappling hook 3-5 m from a boat and slowly retrieving it along with attached plant material, or by diving to the bottom and pulling handfuls of hydrilla from the hydrosol. On each sampling occasion, 1 or 2 40-L buckets were approximately half filled with hydrilla and then transported to a laboratory in water collected from the same location. At the laboratory, stems were haphazardly selected from the collection and their growing tips closely inspected for signs of damage. The sample size varied from 120 to 270 tips ($n = 6$) (Table 1). Growing tips were scored as 'bored' (typically a lateral tunnel bored

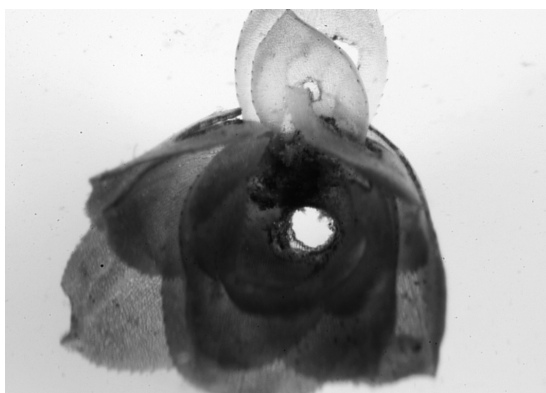


Fig. 2. Example of vertical boring damage in wild hydrilla, with tunneling continuing into the stem.

TABLE 1. SUMMARY OF LABORATORY EXPERIMENTAL CONDITIONS.

Experiment	No of containers; treatment/control	Source of hydrilla for experimental containers	Substrate used in experimental containers	No. of <i>P. adusta</i> added to each treatment container
1	6/2	Lake Tanganyika	mud	10
2	10/2	Lake Tanganyika	mud	12
3	10/2	Lake Tanganyika	mud	10
4	10/2	Lake Tanganyika	mud	22
5	10/2	Aquarium culture	sand	27
6	10/2	Aquarium culture	sand	31
7	9/5	Aquarium culture	sand	7 or 8

through the bud, see Fig. 1), ‘missing’ or ‘intact’. *G*-tests of independence were used to compare the proportions of stems with bored and missing meristems on different sampling dates at each location.

RESULTS

Laboratory Experiments

The number of hydrilla sprigs damaged by *P. adusta* per experiment ranged from 1-9 (1.4-7.5% of exposed stems, 4.1% in all experiments combined), whereas no sprigs were damaged in any of the control containers (Table 2). The majority of damage was to apical meristems, but a few sprigs had damage to stems, or to both stems and apical meristems (Fig. 2). An example of experimental damage to hydrilla by *P. adusta* is shown in Fig. 3. The type of damage was identical to that seen in wild hydrilla from Rumonge (Fig. 1). In addition to burrowing in plant material, in experiment 7, 11 nymphs were found to have constructed burrows made of sand grains and silken secretions that adhered to leaves at the base of stems (Fig. 4). One *P. adusta* nymph was found in a burrow in the apical meristem in each of the second

and last experiments (Figs. 5 and 6). In experiment 6, 2 bored tips (1 in each of 2 treatment containers) were found to contain a single chironomid larva, apparently accidentally introduced into the containers as a result of a pipetting error. The midge larvae were sent to John Epler and he reported that they were third instars and probably *Polypedilum wittei*.

Even though damage was only found in containers receiving *P. adusta*, the number of sprigs with damage was not statistically different between treatment and control containers (Fisher’s exact test *P* values range from 0.10 to 0.75). However, when data were pooled for all experiments, significantly more sprigs were attacked in containers with *P. adusta* (30/750) compared to control containers (0/204) (*P* = 0.0008).

Field Sampling

Hydrilla stems collected at both Rumonge and Magara were damaged with 5-52% of plants having bored apical meristems and 13-57% of stems having lost their growing tips (Table 3). The proportion of bored apical meristems and the proportion of plants lacking growing tips differed between sampling dates at Rumonge (*G* = 69.8, df =

TABLE 2. DAMAGE TO HYDRILLA SPRIGS IN TREATMENT CONTAINERS WITH *P. ADUSTA* NYMPHS¹.

Experiment	Type of damage			
	Apical meristems bored only	Stem bored only	Apical meristems and stems bored	Total stems with damage
1	1	0	0	1
2	3	0	0	3
3	3	0	0	3
4	3	0	2	5
5	2	4	3	9
6	1	1	2	4
7	5	0	0	5
All experiments	18	5	7	30

¹No stems in control containers were damaged.



Fig. 3. An example of the tunneling damage seen in Hydrilla from experimental containers. The perspective of the photograph hides additional leaf damage. Note the similarity to the type of damage seen in field collected hydrilla (Fig. 1).

3, $P < 0.001$ and $G = 78.9$, $df = 3$, $P < 0.001$ for bored tips and missing growing tips, respectively), and at Magara ($G = 12.4$, $df = 1$, $P < 0.0004$ and $G = 10.9$, $df = 1$, $P < 0.001$).

DISCUSSION

The laboratory experiments provide clear evidence that *P. adusta* nymphs occasionally burrow in the tips and stems of hydrilla. The proportion of plants exposed to *P. adusta* that were damaged was low, but 2 nymphs were found inside burrows, and no damage at all was observed in containers receiving no nymphs. The low proportion of stems attacked may have been, at least in part, an artifact of the experimental design. After the last experiment, care was taken to completely inspect plant material and the sand in which it was grown in order to recover as many nymphs as pos-



Fig. 4. *Povilla adusta* burrow made from sand particles and silken material attached to a hydrilla leaf in experimental container.

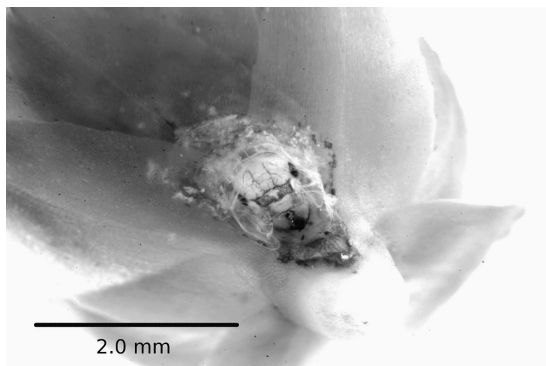


Fig. 5. *Povilla adusta* nymph in a vertically-oriented burrow within the apical meristem of hydrilla from an experimental container. Only the head is visible with the rest of the nymph hidden in the burrow that extends up through the leaves. Note that damage has extended into the stem at the base of the bud. This orientation would produce the damage seen in wild hydrilla as pictured in Fig. 2.

sible. Eleven nymphs were found to have constructed burrows made from sand and silken material attached to the basal leaves of hydrilla. Sand was obviously competing with hydrilla tips as a substrate for the construction of burrows. In our field survey, hydrilla was normally found growing in muddy substrates in Lake Tanganyika and in several Ugandan lakes (Copeland, personal observation). When sand was made artificially available in our experimental containers, *P. adusta* used it to construct burrows. In natural areas where hydrilla is found, the fine particulates of mud may provide poor material for burrow construction, and thus, early-instar *P. adusta* nymphs may have no alternative but to burrow into hydrilla tips or other available plant material. Additionally, the mud collected from near Bujumbura, the densely populated capital city of Burundi, may have had a deleterious effect on *P.*

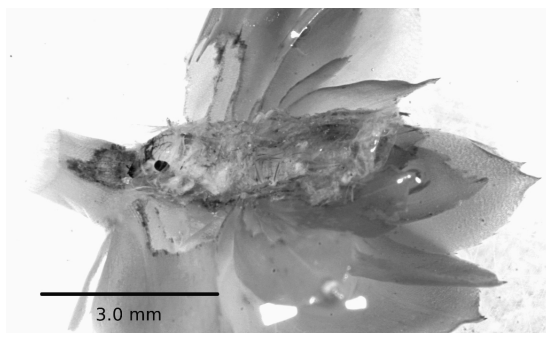


Fig. 6. Same individual as in Fig. 5 shown in burrow exposed by folding back the damaged hydrilla leaves. Note the damage exhibited by several leaves.

TABLE 3. PROPORTIONS OF HYDRILLA STEMS WITH BORED, MISSING AND INTACT APICAL MERISTEMS FROM SAMPLES COLLECTED AT 2 LOCATIONS IN LAKE TANGANYIKA, BURUNDI.

Location	Date	Number of stems	Proportion with bored apical meristem	Proportion missing apical meristem	Proportion intact
Rumonge	6-II-2010	270	0.23	0.57	0.21
	19-II-2010	120	0.22	0.48	0.30
	7-XI-2010	179	0.22	0.46	0.32
	16-III-2011	128	0.52	0.14	0.34
Magara	6-II-2010	270	0.17	0.27	0.56
	19-II-2010	120	0.05	0.13	0.83
Total		1087	0.24	0.37	0.39

adusta nymphs. During 4 years of repeated sampling at the Bujumbura site, no bored growing tips or *P. adusta* nymphs were observed (R. Copeland, personal observation). Immature mayflies in general are known to be sensitive to water quality, and have been used as bio-indicators (Bonada et al. 2006). Finally, the abiotic conditions in experimental containers, such as the amount of dissolved oxygen, pH and temperature, may not have been optimal for nymphal activity and survival. Petr (1973) found that the distributions of *P. adusta* in Lake Volta in Ghana and Lake George in Uganda were related to pH and the concentration of dissolved oxygen.

Field surveys demonstrated that the level of damage to hydrilla from *P. adusta* in Lake Tanganyika can be substantial, with as many as 52% of tips bored. Boring in growing tips, and particularly stems, may conceivably lead to tip abscission, suggesting that at least some missing apical meristems may also have resulted from damage inflicted by *P. adusta*. Pemberton (1980) also found that a high proportion (65-80%) of the stems from Lake Tanganyika were damaged, having either bored or missing stems.

The difference in the levels of tip-boring damage we observed between sampling dates suggests a temporal variation to plant damage from *P. adusta*. A number of studies have investigated the emergence pattern of adult mayflies. Hartland-Rowe (1958) showed that emergence in Lake Victoria was closely linked to the lunar cycle with most adults emerging 2 nights after the full moon, whereas in several other East African lakes, there was only a weak or indiscernible pattern of emergence. In West Africa, Corbett (1974) found that emergence in a lake in Cameroon was closely synchronized with the lunar cycle occurring 4 d after the full moon, but the relationship was weaker in other lakes. Based on the variation in damage to hydrilla between sampling dates in Lake Tanganyika, it appears that *P. adusta* populations may exhibit a degree of synchrony in their development.

Although Pemberton (1980) and Markham (1985) concluded that the burrows in hydrilla tips in Lake Tanganyika were caused by *Polypedilum* spp., their conclusions were based on the observation that bored tips occasionally were found to contain midge larvae. However, Pemberton (1980) indicated that the damage was usually observed in the absence of midge larvae. Based on our findings, we suggest that midge larvae occasionally colonize burrows that have been constructed by *P. adusta* and later abandoned, as occurred in 1 of our laboratory experiments when 2 burrows were found occupied by third instar midge larvae, probably *P. wittei*. The behavior of one of these larvae was observed by one of us (RSC) for a period of about 15 min. The larva made repeated forays out of the burrow to scrape the surface of hydrilla leaves, always remaining anchored by its hind prolegs to the inside of the burrow. Herbivory was not observed. Moreover, both *P. wittei* and *P. dewulfi* have mouthparts characteristic of collector-gatherer-scraper species in the genus, as opposed to true leaf or stem-miners, which typically have more worn mouthparts (John Epler, personal communication).

Nonetheless, in laboratory experiments both Pemberton (1980) and Markham (1985) reported burrowing by chironomid larvae into undamaged hydrilla, with destruction of the apical meristem (Pemberton 1980). Therefore, we do not rule out possible tunneling damage by chironomids. In our final experiment, we intended to use chironomid larvae in a set of our treatment containers. However, this was not possible because at that time midge larvae were nearly absent from Rumonge hydrilla. That 52% of hydrilla tips at Rumonge had boring damage during this same time interval (Table 3) suggests that most or all of the damage was due to the activity of *P. adusta* nymphs, not midge larvae.

It is somewhat surprising that we did not find any *P. adusta* nymphs in burrows in field-collected hydrilla stems, and only 2 nymphs were found in burrows during examination of labora-

tory exposed sprigs, strongly suggesting that when disturbed, nymphs leave their burrows. Pemberton (1980) speculated that fish grazing was responsible for missing apical meristems, which could explain *P. adusta* nymphal behavior—they may quickly abandon burrows when disturbed to avoid predation.

This study demonstrated that *P. adusta* nymphs bore in hydrilla apical meristems and stems. The damage they cause may be one factor in the regulation of hydrilla in East African lakes, where it is not considered to be a weed (Pemberton 1980; Verkleij et al. 1983; Gidudu et al. 2011). One reason for the lack of weediness in Africa is that hydrilla rarely reaches the surface, and therefore is less likely to shade out other plants, interfere with navigation, disrupt water flow and influence water quality. Burrowing in the meristem by *P. adusta* may break apical dominance in hydrilla and cause plants to branch before they reach the water surface, as has been shown to occur in hydrilla attacked by the phytophagous midge, *Cricotopus lebetis* Sublette (Cuda et al. 2002, 2011). Even though *P. adusta* causes significant levels of damage to hydrilla in Lake Tanganyika, it cannot be considered for use as a biological control agent as it is not a herbivore and will utilize a wide range of plant and inorganic materials to construct burrows. The *Polypedilum* spp. earlier thought to cause substantial damage to hydrilla in Lake Tanganyika were not associated with damage in our study, and we recommend that they receive no further attention as candidate biological control agents of hydrilla.

ACKNOWLEDGMENTS

We thank the Director General, Institut National pour l'Environnement et la Conservation de la Nature, Burundi, for facilitating the project. Mathias Nyabenda provided excellent support in the laboratory and the field. John Epler provided identifications of chironomid larvae. We also thank the following agencies for providing financial support: the Florida Fish and Wildlife Conservation Commission, the South Florida Water Management District, the Saint Johns Water Management District and Osceola County, Florida.

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