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First record and ecological features of *Goeldichironomus petiolicola* (Diptera: Chironomidae) mining *Eichhornia crassipes* in the Middle Paraná River floodplain, Argentina

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Primer registro y aspectos ecológicos de *Goeldichironomus petiolicola* (Diptera: Chironomidae) minador de *Eichhornia crassipes* en la llanura aluvial del río Paraná Medio, Argentina

■ **RESUMEN.** Se presenta el primer registro para Argentina de *Goeldichironomus petiolicola* Trivinho-Strixino & Strixino, especie cuyos estadios inmaduros minan dentro de los pecíolos de *Eichhornia crassipes* (Martius) Solms, en humedales del río Paraná Medio. Las larvas y pupas de *G. petiolicola* fueron recolectadas estacionalmente durante 2005, de los stands de *E. crassipes* y en muestras de sedimento en diferentes ambientes de la llanura. Ejemplares de la especie fueron criados en laboratorio a fin de completar su ciclo de vida. *G. petiolicola* fue el quironómido más abundante que habita en el interior de los pecíolos de *E. crassipes*, se registraron los estadios de larva III y IV y pupa durante todas las estaciones. No fueron registrados los estadios larvales I y II. Las mayores densidades medias de *G. petiolicola* fueron obtenidas en otoño-invierno (588,5 ind.kg⁻¹ dw - 778,7 ind.kg⁻¹ dw), esto sugiere que presentan mejores condiciones para la supervivencia, lo que permite a los individuos obtener un desarrollo más lento hasta el próximo período de inundación, con mayores temperaturas. El valor calculado de r (Dyar) fue 1,66. *G. petiolicola* mina dentro de los pecíolos de *E. crassipes*, principalmente, durante el inicio del proceso de senescencia del vegetal, por lo que no provoca daños importantes.

PALABRAS CLAVE. Chironomidae. Minador de *Eichhornia crassipes*. Ecología larval. Humedales.

■ **ABSTRACT.** The first records from Argentina of larvae of *Goeldichironomus petiolicola* Trivinho-Strixino & Strixino mining in *Eichhornia crassipes* (Martius) Solms petioles in wetlands of the Paraná River floodplain were analyzed. *G. petiolicola* larvae and pupae were collected seasonally during 2005 from stands of *E. crassipes* and bottom sediment samples in floodplain habitats. Larvae inside *E. crassipes* petioles were reared

with the purpose of obtaining their complete life cycle. *G. petiolicola* was the most abundant chironomid species living inside *E. crassipes* petioles; the III and IV instar larvae and pupae were found during all the seasons while the I and II instar larvae were not found. The highest average densities of *G. petiolicola* larvae inside the *E. crassipes* petioles were recorded in Autumn-Winter (588.5 ind.kg⁻¹ dw - 778.7 ind.kg⁻¹ dw). This suggests that they have better conditions for survival, allowing the individuals to develop more slowly until the next flooded period with higher temperatures. The *r* (Dyar) value obtained was 1.66. We conclude that *G. petiolicola* mines into *E. crassipes* petioles mainly when the plants begin their senescence process and does not cause important damage to them.

KEY WORDS. Chironomidae. *Eichhornia crassipes* miner. Larval ecology. Wetlands.

INTRODUCTION

The immature stages of Chironomidae are one of the most abundant and diverse group of insects in freshwater habitats and live associated with benthic, periphyton and macrophyte communities (Coffman & Ferrington, 1984; Fittkau, 1986; Pinder, 1995; Paggi, 2009). They play an important role in the aquatic food webs, in the decomposition processes of organic matter, in nutrient cycling and as bioindicators of environmental conditions (Armitage *et al.*, 1995; Paggi, 1998; Henriques-Oliveira *et al.*, 2003). Despite the diverse ecological roles played by chironomids, as well as their widespread occurrence in freshwater ecosystems, surprisingly few studies have analyzed their ecology and population dynamics (Trivinho-Strixino & Strixino, 1982; Corbi & Trivinho-Strixino, 2006).

Goeldichironomus Fittkau is a predominantly Neotropical genus with 11 species described. *Goeldichironomus petiolicola* has been recently described by Trivinho-Strixino & Strixino (2005) from Brazil, where most of the species in this genus were reported (Reiss, 1974; Spies & Reiss, 1996; Trivinho-Strixino & Strixino, 1989, 1991, 1998).

The larvae of *Goeldichironomus* live in drifting vegetation and bottom sediments of small standing water bodies (Pinder & Reiss, 1983). In addition, two species of this

genus have been recorded mining inside macrophytes, *G. holoprasinus* (Goeldi) in *E. crassipes* (Bennett & Zwolfer, 1968) and *G. petiolicola* in *Eichhornia azurea* (Swartz) Kunth and *Pontederia cordata* L. var. *cordata* (Trivinho-Strixino & Strixino, 2005).

Eichhornia crassipes is a native of Brazil and is one of the most abundant macrophytes in the Paraná River floodplain, mainly in floodplain lakes, where it also comprises the greatest biomass of these habitats (Lallana, 1980; Sabattini & Lallana, 2007). Due to its adaptability, this species has colonized temperate and tropical water bodies around the world. This is an invasive plant that has generated important economic losses and damages in natural and human-made habitats. Because of this, several studies concerning invertebrates that might be used to control the populations of this plant have been done (Silveira Guido & Perkins, 1975; Deloach & Cordo, 1976; Poi de Neiff *et al.*, 1977; Casco & Poi de Neiff, 1998; Heard & Winterton, 2000; Adis & Junk, 2003; Poi de Neiff & Casco, 2003). In the present study, *G. petiolicola* is recorded for the first time from Argentina, its larvae mining *E. crassipes* petioles in floodplain wetlands of the Middle Paraná River system. The immature individuals found inside the plant petioles were measured, reared and some aspects of their life cycle were analyzed with the purpose to improve information about *G. petiolicola* biology and ecology features



Fig. 1. Study area located in the Paraná River floodplain, Argentina. 1: Paraná City, 2: Santa Fe City.

that can be useful to evaluate the degree of damage caused in *E. crassipes*.

MATERIAL AND METHODS

The sampling station selected was a shallow lake of the Middle Paraná River floodplain (31° 43' S - 60° 39' W) (Fig.1). *Eichhornia crassipes*, *Salvinia herzogii* de la Sota, *Azolla caroliniana* Willdenow, *Pistia stratiotes* L., *Echinochloa polystachya* (Kunth) Hitchcock, *Polygonum acuminatum* Kunth, *Eleocharis* R. Brown spp. and *Paspalum repens* Bergius were the dominant macrophytes in this habitat.

Stands of *E. crassipes* and bottom sediment samples were taken seasonally during 2005 to collect *G. petiolicola* larvae and pupae, including the high water period of the Paraná River in January-February and the low water period in August-September. Three samples of *E. crassipes* were taken in different stands using a square method (1m²) and three samples of bottom sediment were collected with an Ekman grab of 225 cm², filtered through a 200 µm sieve and fixed in 10% formaldehyde in the field. Water temperature, pH, conductivity (with water probe Horiba U-10) and depth were measured on each sampling date. Air temperature (standard thermometer) was also measured because the chironomids were sampled inside the aerial plant petioles.

In the laboratory, all chironomid larvae and pupae were removed from the interior of *E. crassipes* petioles and bottom sediment samples under a stereoscopic microscope (4x) and preserved in alcohol 70%. Total body length (measured from the anterior margin of the cephalic capsule) and cephalic capsule width (maximum ventral width of the cephalic capsule measured transverse to the major axis of the body) of *G. petiolicola* larvae were measured with a micrometer scale under an optical microscope. The larvae were separated into instars according to the relationship between cephalic capsule width and total body length and Spearman *Rho* correlation was estimated. In order to determine the growth proportion (*r*) between recorded larval instars, the Dyar proportion was calculated (Dyar, 1890).

Dry weight of petiole biomass was obtained by drying material at 60 °C down to a constant weight. The larval and pupal densities values were expressed as ind. kg⁻¹ *E. crassipes* petioles biomass dry weight.

To rear and obtain adult specimens of *G. petiolicola*, larvae inside *E. crassipes* petioles were put in separate vials at room temperature during May of 2005 (11.5 °C - 23.4 °C) following the procedures described by Paggi (2009). The reared and collected material was mounted on slides with Euparal® or Hoyer's medium and was measured under an optical microscope following the procedures described by Paggi (2009).

Table I. Environmental parameters recorded in each sampling date.

	Summer Jan-13	Autumn Apr-08	Winter Jul-07	Spring Oct-15
Air temperature (°C)	33.8	27.5	14.0	18.8
Water temperature (°C)	29.0	24.5	15.0	18.0
pH	7.2	7.4	7.4	7.4
Conductivity $\mu\text{S}\cdot\text{cm}^{-1}$	90	105	80	80
Depth (m)	2.21	2.29	2.70	2.50

Table II. Average densities (\pm SD) (ind.kg⁻¹), seasonal and total percentage frequency of Chironomidae (Diptera) larvae living inside of *Eichhornia crassipes* petioles from Summer to Spring 2005.

Taxa of Chironomini	Summer	Autumn	Winter	Spring	Total
<i>Goeldichironomus petiolicola</i>	101.3 \pm 24.3 (86.13%)	589.6 \pm 673.8 (98.9%)	778.6 \pm 251.2 (95.11%)	303.1 \pm 311.3 (95.94%)	94%
<i>Endotribelos</i> Grodhaus sp.	13.8 \pm 12.6 (11.74%)	3.9 \pm 3.5 (0.66%)	30.8 \pm 11.8 (3.77%)	7.2 \pm 9.5 (3.02%)	4.8%
<i>Asheum</i> Sublette & Sublette sp.	2.5 \pm 2.7 (2.13%)	2.6 \pm 3.1 (0.44%)	3.7 \pm 3.3 (0.45%)	3.6 \pm 6.3 (0.67%)	0.93%
<i>Polypedilum</i> Kieffer sp.	-	-	5.5 \pm 4.6 (0.67%)	1.2 \pm 2.1 (0.37%)	0.27%

The specimens were identified as *G. petiolicola* according to Trivinho-Strixino & Strixino (2005). Voucher specimens were deposited at the Instituto de Limnología Dr. Raúl A. Ringuelet (La Plata, Argentina).

RESULTS AND DISCUSSION

Macrophyte substratum

The air temperature in the macrophyte stands in Summer-Autumn was higher than the water temperature. The air and water temperatures were very similar in Spring and Winter. The water depth varied between 2.21 m and 2.70 m according to the hydrologic regime and the water pH and conductivity were similar in all seasons (Table I).

Goeldichironomus petiolicola was the most abundant chironomid species living inside *E. crassipes* petioles (86.1% - 98.9%). *Endotribelos* Grodhaus sp., *Asheum* Sublette & Sublette sp. and *Polypedilum* Kieffer sp. were also recorded inside the petioles, but in low densities (Table II).

Two clearly separable groups of *G.*

petiolicola (Fig. 2), corresponding to third (III) and fourth (IV) instar larvae (N= 371), were observed when comparing the cephalic capsule width and the total body length. The maximum body length and cephalic capsule width of the III and IV instar larvae were 4.51 mm and 3.22 mm and 9.05 mm and 5.62 mm, respectively (Table III). The Spearman correlation between cephalic capsule width and total body length was Rho : 0.714 ($p < 0.01$).

The maximum total body length of the single IV instar larva specimen from Brazil (Trivinho-Strixino & Strixino, 2005) was smaller (8.2 mm) than the specimens from Argentina.

Only III and IV instar larvae of *G. petiolicola* were found during all the seasons (except only III instar in Summer) mining the petioles of *E. crassipes*; while I and II instar larvae were not found during the study year. We can assume that they mine the petioles after the II larval instar and they are not easily sampled by ordinary sampling methods because of their small size (Pinder, 1995).

The r (Dyar) value obtained was 1.66. This value was higher than reported for *G.*

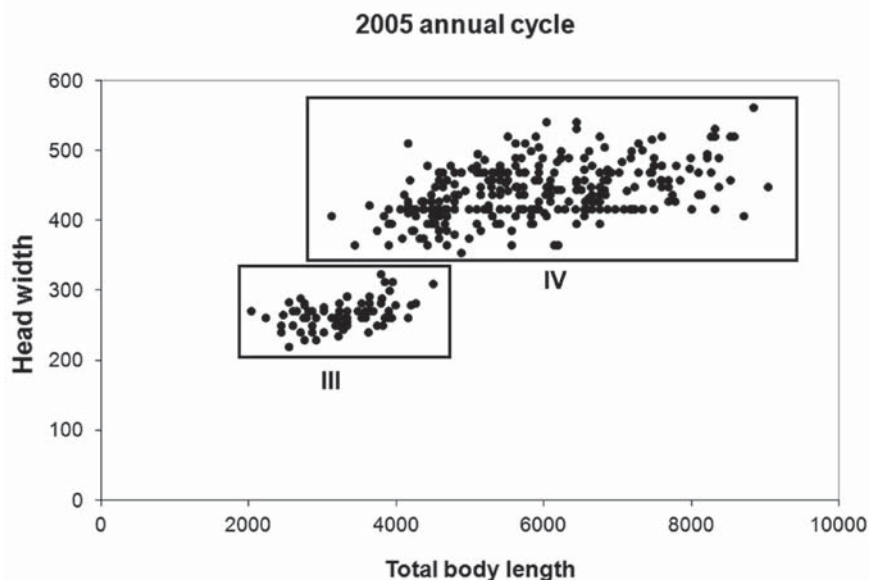


Fig. 2. Relation between cephalic capsule width (μm) and total body length (μm) of III and IV larval instars of *Goeldichironomus petiolicola* mining inside *Eichhornia crassipes* petioles recorded during the annual cycle of 2005.

Table III. Body length minimum, maximum and average (\pm SD) and head width size recorded for III and IV instar larvae of *Goeldichironomus petiolicola*. All measurements are in μm .

	Summer	Autumn	Winter	Spring
III instar larvae				
Cephalic capsule width				
Min-max	-	218.4-322.4	234.0-312.0	239.2-312.0
Average \pm SD	-	269.64 \pm 22.72	260.70 \pm 17.66	268.40 \pm 18.69
Body length	-	2444-4506	2236-4264	2745-3900
IV instar larvae				
Cephalic capsule width				
Min-max	364.0-561.6	374.4-520.0	353.6-540.8	384.8-520.0
Average \pm SD	444.11 \pm 53.75	440.68 \pm 33.52	438.09 \pm 38.80	448.75 \pm 32.92
Body length	3900- 8840	4160-9050	3120-7488	3900-8528

holoprasinus (1.64) (Zilli *et al.*, 2009) and *G. luridus* Trivinho-Strixino & Strixino, 2005 (1.62) and lower than *r* value reported for *G. maculatus* Trivinho-Strixino & Strixino, 1991 (1.75) (Corbi & Trivinho-Strixino, 2006). The use of the Dyar *r* value is important for insects with immature stages on macrophytes like *G. petiolicola*, because it is very difficult to find the first instars in the field (they are most likely planktonic); the size of I and II

instars can be estimated through the *r* value obtained (Strixino, 1973).

The larvae and pupae of *G. petiolicola* live in shelters with two openings just below the petiole's epidermis; their sizes are coincident with the maximum body length of approximately 1 cm (Trivinho-Strixino & Strixino, 2005) (Fig. 3). This particular behavior makes this species very difficult to rear under laboratory conditions; for this

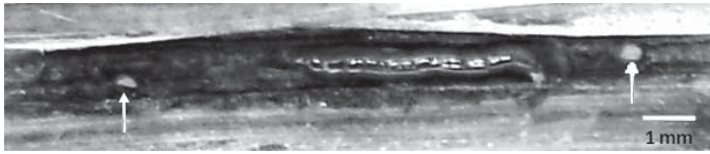


Fig. 3. Larva of *Goeldichironomus petiolicola* inside a shelter in *Eichhornia crassipes* petiole. The arrows indicate the two tube openings.

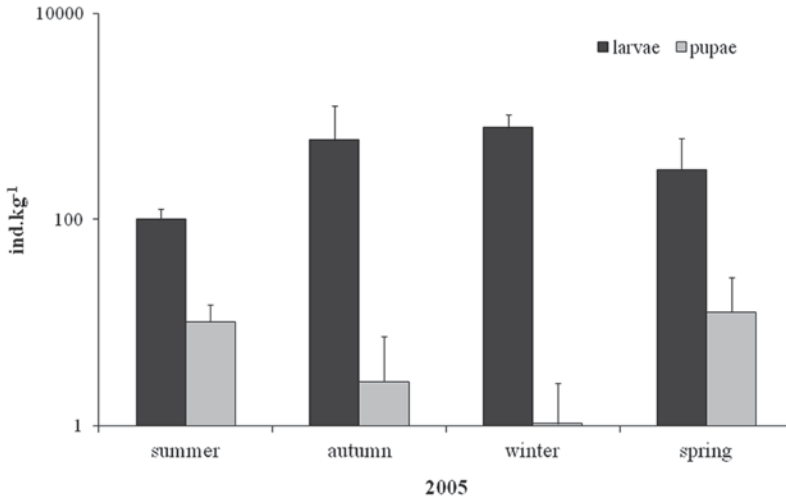


Fig. 4. Density (ind.kg⁻¹) of larvae and pupae of *Goeldichironomus petiolicola* inside *Eichhornia crassipes* petioles recorded during all seasons of 2005.

reason we could not define how many days it takes to complete the life cycle. However, there are data for the life cycles of other *Goeldichironomus* species. In laboratory conditions, the average life cycle of *G. holoprasinus* (18 °C-33 °C) was 18 days (Zilli *et al.*, 2009); for *G. maculatus* and *G. luridus* (21 °C-26 °C) it was 28 and 20 days, respectively (Corbi & Trivinho-Strixino, 2006).

The presence of larvae and pupae during all seasons indicates that this species can reproduce throughout the year with overlapping generations (Tokeshi, 1995). The highest average densities of *G. petiolicola* larvae in *E. crassipes* petioles were recorded in Autumn-Winter (588.5 ind.kg⁻¹ dw - 778.7 ind.kg⁻¹ dw) (Fig. 4), suggesting that they have better conditions to survive allowing the individuals to get slower development until the next flooded period with higher temperatures.

The highest pupal density values were

found in Spring-Summer (10.3 ind.kg⁻¹ dw - 12.8 ind.kg⁻¹ dw) (Fig. 4) and were in direct relationship with higher water and air temperatures for the year, similar to Chironomini in temperate zones as stated by Coffman (1973). It is common among multivoltine species that the first Spring emergences are stronger, in terms of growth and development, than later emergences which coincide with rising temperatures and increasing photoperiod in Spring (Tokeshi, 1995).

Goeldichironomus petiolicola has been described as a facultative phytophage, feeding on seston and using plant material only as a substratum for mining. Thus, the larvae consume plant tissue incidentally when building their shelters (Trivinho-Strixino & Strixino, 2005). We observed that *G. petiolicola* mines into *E. crassipes* petioles mainly when the plants begin their senescence and decomposition processes (Autumn-Winter) and the water content (%)

in vegetal tissues is higher (Lallana, 1980), but we couldn't find signs of serious damage produced by chironomids.

Bottom sediments

In the present study larvae and pupae of *G. petiolicola* were not found in bottom sediment samples. There was a record of specimens of *G. petiolicola* in bottom sediment, but they were inside decomposing tissues of *E. crassipes* petioles, in the marginal fluvial wetland of the Middle Paraná secondary channel (Montalto, unpub.). The average density of *G. petiolicola* larvae in decomposing tissues of *E. crassipes* petioles in marginal fluvial wetland was 254 ind.m⁻² (Montalto, unpub.). This density was lower than the ones found for other *Goeldichironomus* species that live in bottom sediments of freshwater habitats; *G. maculatus* had reported densities of 475-483 ind.m⁻² in sandy lentic habitats from Brazil (Corbi & Trivinho-Strixino, 2006).

According to Montalto & Paggi (2006) and Montalto (unpub.), marginal wetlands are subject to fluctuating conditions. The invertebrates that live in these temporary wetlands are adapted to withstand such fluctuating environments, featuring rapid changes and periodical situations of desiccation. Thus, during low water levels when *E. crassipes* plants drop down to the bottom sediment, immature individuals that live inside petioles are able to survive desiccation periods and emerge when their development is completed. The fact that *G. petiolicola* larvae and pupae were not found in bottom sediment samples free of decomposing macrophytes leads us to assume that this species is exclusively associated with macrophytes. Nevertheless, more studies are needed.

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