Short note [Nota corta]

FIRST RECORD OF EPIBIONT *Sigara* sp. (Corixidae) IN CRAYFISH *Procambarus* (*Austrocambarus*) *llamasi* FROM SOUTHEASTERN MEXICO †

[PRIMER REGISTRO DEL EPIBIONTE *Sigara sp*. (Corixicidae) EN EL CANGREJO DE RIO *Procambarus* (*Austrocambarus*) *llamasi* DEL SURESTE DE MÉXICO]

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SUMMARY

Background. In Mexico, the biology of *Procambarus* has been more studied than other cambarid species due to its diversity and potential use in aquaculture. Fecundity, egg development, and growth under laboratory conditions have been evaluated of the *Procambarus* (*Austrocambarus*) *llamasi*. The effect of density and sex ratio on the development and spawning of gonads and growth of juveniles fed different commercial agricultural and aquaculture feeds has also been studied. There are no records of epibionts infesting this crayfish. The primary objective of this study is to determine the infestation and epibiont identification rates of the Corixidae family and the damage caused to the crayfish. **Methodology.** To determine infestation indexes were calculated: the mean intensity (IM) is the average parasite species in infected organisms (MI = n. Total parasites / n. Infected hosts); abundance (AB) is the average number of parasites per host examined (AB = total number of parasites / n. hosts examined), and prevalence (P) indicates the percentage of organisms parasitized by a species of parasites (P = n .infested hosts / n. hosts examined x 100). **Results.** Of the shrimp analyzed, 76 were infested with 2655 aquatic insect eggs corresponding to 34.93 intensity; 18.18 abundance, and 52.05% prevalence. As far as crayfish sex, infestation occurred in 39% (n=30) of females and 61% (n=46) of males. The aquatic insect in this study belongs to the genus *Sigara* sp., being the only species registered in the crayfish. **Implications.** When the parasite egg settles near the crab eye region, it is likely to cause visual problems or partial blindness; on the other hand, it could cause a deterioration in the health of the populations of these crabs. **Conclusion.** The eggs located on the cuticular surface of the cephalothorax of *Procambarus* (*Austrocambarus*) *llamasi* correspond to insect the genus *Sigara* sp. Half of the studied population of the crayfish is infested by this epibiont. According to the results obtained it was determined that the eggs on the shrimp's cuticular surface cause cuticle damage due to cementation on its shell. Eggs can also cause esthetic damage affecting marketing for human consumption and make shrimp overweight due to the egg mass.

Keywords: bug; crayfish; indexes; parasite.

† S*ubmitted August 9, 2020 – Accepted November 23, 2020.*
ISSN: 1870-0462.

RESUMEN

Antecedentes: En México, la biología de *Procambarus* ha sido más estudiada que otras especies de cambáridos debido a su diversidad y uso potencial en la acuicultura. Del cangrejo de río *Procambarus* (*Austrocambarus*) *llamasi* se ha evaluado la fecundidad, el desarrollo de huevos y el crecimiento en condiciones de laboratorio. Asimismo, se ha estudiado el efecto de la densidad y la proporción de sexos en el desarrollo y desove de las gónadas y el crecimiento de juveniles alimentados con diferentes alimentos comerciales agrícolas y acuícolas. No hay registros de epibiontes que infesten este cangrejo de río. El objetivo de este estudio fue determinar los índices de infestación e identificación de epibionte de la familia Corixidae y el daño causado al cangrejo de río. **Metodología:** Para determinar los índices de infestación se calcularon: la intensidad media (IM) es la especie parásita promedio en organismos infectados (MI = n. parásitos totales / n. huéspedes infectados); la abundancia (AB) es el número promedio de parásitos por huésped examinado (AB = número total de parásitos / n. huéspedes examinados), y la prevalencia (P) indica el porcentaje de organismos parasitados por una especie de parásito (P = n. huéspedes infectados / n. hosts examinados x 100) e identificando, contando y midiendo huevos y ninfas infestando. **Resultados:** De los camarones analizados, 76 estaban infestados con 2655 huevos de insectos acuáticos, lo que corresponde a 34.93 de intensidad; 18.18 abundancia, y 52.05% de prevalencia. En cuanto al sexo de los cangrejos, la infestación ocurrió en el 39% (n = 30) de las hembras y el 61% (n = 46) de los machos. El insecto acuático en este estudio pertenece a *Sigara* sp, siendo la única especie registrada en el cangrejo de agua dulce. **Implicaciones.** Cuando el huevo del parásito se fija cerca de la región ocular de los cangrejos probablemente causa problemas visuales o ceguera parcial; por otro lado podría provocar un deterioro en la salud de las poblaciones de estos cangrejo. **Conclusión:** Los huevos localizados en la superficie cuticular del cefalotórax de *Procambarus* (*Austrocambarus*) *llamasi* corresponden a insectos del género *Sigara* sp. La mitad de la población estudiada del cangrejo de agua dulce esta infestada por este epibionte. De acuerdo a los resultados obtenidos, se determinó que los huevos en la superficie cuticular del camarón causan daños en la cutícula causado por la cementación en su caparazón. Los huevos también pueden causar daños estéticos que afectan la comercialización para el consumo humano y hacer que los camarones tengan sobrepeso debido a la masa de los huevos.

Palabras claves: insecto; cangrejo de río; índices; parásito.

INTRODUCTION

Within the family Cambaridae, the genus *Procambarus* is especially important due to the number of species it includes and its wide distribution in the American Continent (Torres, Álvarez and Botello, 2013). The crayfish *Procambarus* (*Austrocambarus*) *llamasi* (Villalobos-Figueroa, 1955) is geographically distributed in North America, from southern Canada, the eastern portion of the United States, and southeastern Mexico, in epicontinental water bodies, including lakes, swamps, and floodplains, which may be relatively isolated (Villalobos-Figueroa, 1955, Hobbs, 1989; Álvarez, *et al*., 2014; Rodríguez-Serna *et al.,* 2002; Barba, Juárez-Flores and Estrada, 2010; Barba-Macias *et al.,* 2015). This cambarid, like other species in the family, builds burrows in flood zones or swamps, which are very common in southern Mexico (Álvarez, Villalobos and Robles, 2005).

In Mexico, the biology of *Procambarus* has been more studied than the biology of other Cambarids because of its diversity and potential use in aquaculture (Indy *et al.,* 2010). These studies include fecundity, egg development and, growth of juvenile crayfish *Procambarus (Austrocambarus) llamasi* under laboratory conditions, the effect of density and sex ratio on gonad development and spawning and the growth of juvenile fed different farm and aquaculture commercial foods (Rodriguez-Serna, *et al*., 2000; Rodriguez-Serna *et al*, 2002; Carmona *et al.*, 2004; Rodriguez-Serna *et al*, 2010; Carmona-Osalde *et al.*, 2015).

The main parasites in wild or cultured shrimp are *Gregarines*, *Microsporidia*, *Haplosporidia*, *Epicommensals, Epistilis* and epibionts (algae, filamentous bacteria, and protozoan) and Metazoan (trematodes and nematodes) (Olivas, Cáceres and Vázquez *et al*., 2010; Cuéllar-Anjel, 2013; Galaviz *et al*., 2016). The epibionts that have been reported in crayfish are protozoa *Epistylis* sp., *Vorticella* sp., and insect eggs (Smith, Sandifer and Manzi, 1979; Vasquez *et al*., 2005, Cabrera and Rubio, 2012). However, there are no records of parasites affecting or symbiotically interacting with the crayfish *P. llamasi*, except for a case of the epibiont *Sigara* sp. found on the cephalothorax of this host located in water bodies in southeastern, Mexico. Therefore, the objective of this study was to determine the infestation index and morphometric characteristics of the epibiont *Sigara* sp., and the damage caused to the organisms.

MATERIALS AND METHODS

Area of study**:** The research was conducted in November 2018 in a 30 cm deep mud pool, in Ejido Los Rieles de San José, 17°21'46" N and 91°22'07" W, 15 km from the municipality of Tenosique, Tabasco, Mexico.

Determining and identifying sex: *P. llamasi* organisms were manually caught with circular baskets, measured (mm) with a conventional ruler, and weighed with a 0.01 g-7000 g digital balance to obtain the length (L) and weight (W) of each specimen. Subsequently, figures were recorded in an Excel database to determine the minimum and maximum data as well as the average and standard deviation by size frequency.

Looking for epibionts: A total of 146 specimens of *P. llamasi* were checked for insect eggs (epibiont) on their anatomical structures: antennae, rostrum, cephalothorax, abdomen, telson, uropods, maxillipeds, pereiopods, and pleopods (Johnson, 1995).

Identification, counting and measurement of eggs and nymphs that infest *P. llamasi:* Insect eggs were counted using a stereoscopic microscope and a manual counter and measured with an optical microscope and an adapted micrometer to obtain the following measurements in mm: total egg length (Tel), egg length to peduncle (Elp), total egg width (Tew), peduncle egg length (Pel), and base egg length (Bel). Also, minimum-maximum, average, and standard deviation measures were obtained.

Obtaining *Sigara* sp., specimens: After shrimp were checked and epibiont eggs were counted, nymphs and juveniles were bred to produce adults to be identified. To this end, shrimp infested with epibiont eggs were kept inside a 45 cm x 20 cm x 15 cm fish tank. *Sigara* sp. specimens were added to the University of Tabasco Insect Collection (CIUT) of the Academic Division of Biological Sciences of Universidad Juárez Autónoma de Tabasco. The epibiont's taxonomic classification was based on the morphological characteristics described by Triplehorn and Johnson (2005); Melo and Scheibler (2011) and Melo (2014).

Calculating infestation indexes: Mean intensity (MI) is the average parasite species in infected organisms (MI= n. total parasites / n. infected hosts); abundance (AB) is the average number of parasites by the hosts examined (AB= n. total of parasites / n. hosts examined), and prevalence (P) indicates the percentage of organisms parasitized by a parasites species (P = n. infested hosts / n. examined hosts x 100). Infestation indexes followed the criteria proposed by Margolis et al. (1982). To determine the relationship between the length (cm) of the crayfish and the mean intensity infection, as well as with the abundance and prevalence; coefficient de correlation Spearman (rs) analyses were applied. All the statistical analyses were carried out with a significance of $p<0.05$.

RESULTS AND DISCUSSION

In this study, we took a closer look at the epibiont group that infests *Procambarus llamasi* in Tabasco, Mexico, and was determined to belong to the genus *Sigara* sp. (Corixidae). Of the total shrimp checked (n=146), 62 were female and 84 were male. L was generally represented by 3-9.5 cm (6.25 ± 1.25 cm) shrimp with a W of 0.49-6.90 g (2.86 ± 1.32 g). Of the shrimp analyzed, 76 were infested with 2655 aquatic insect eggs corresponding to a 34.93 intensity, 18.18 abundance, and 52.05% prevalence. When categorizing shrimp by the size of 1.0 cm, these indexes were higher: between 7.0-8.9 cm L, 40.36 intensity, 24.00 abundance, and 59.46% prevalence (Table 1). *P. llamasi* serves as a substrate for laying the eggs of *Sigara* sp., and the amount of laying may be conditioned to the reproductive state of the females of this insect and at the same time to environmental ecological regulations, stories such as the type of laying substrate, coverage, size and stability of the plant and the ecosystem (Goula and Mata 2015). Taking into consideration shrimp sex, infestation occurred in 39% (n=30) of females and 61% (n= 46) of males. Regarding infestation indexes in *P. llamasi* females and males, they differ from those recorded in females (52.2%) and males (5.9%) in *M. rosenbergii* in South Carolina and from first records of *Ramphocorixa* sp. in Mexico in 1987, whose prevalence ranges from as low as 2% increasing over time to 90% on aquaculture farms (Smith, Sandifer and Manzi 1979; Johnson, 1995; Vásquez *et al*., 2005).

Aquatic insect eggs were specifically located attached to the surface of the cephalothorax of the shrimp (Figure 1 a-b). Eggs were oval-shaped and white (Figure1f). Their Tel was 0.078-0.110 mm (0.099 ± 0.007 mm), Elp was 0.068 – 0.095 mm (0.088 ± 0.006 mm), and Tew was 0.035 - 0.073 mm (0.044 ± 0.007 mm). They also had a peduncle, which holds the eggs, whose Pel was 0.003 - 0.005 mm (0.004 ± 0.001mm). A crater-shaped cementing base with a Bel of 0.018 – 0.040 mm (0.029 ± 0.005 mm) was observed when eggs were removed (Figure 1 c-e). Inside the eggs and through their shells, embryos were observed having optical vesicles and joints (appendices) and being close to hatch (Figure 1 g-h). In this research, this insect egg was taxonomically located in the Corixidae family of the genus *Sigara* sp. (Figure 2). Species from this aquatic insect family (i.e. *Ramphocorixa* sp. and *R. acuminata*) have been reported to infest other shrimp species (*Macrobrachium rosenbergii* and *M. tenellum*)

Table 1. ***Sigara*** **sp. infestation rate in** ***P. llamasi*** **in Tabasco, Mexico.**

Size (cm)	TS	PS	PN	Intensity	Abundance	Prevalence (%)
3.0-4.9	16	2	9	4.50	0.56	12.50
5.0-6.9	89	50	1753	35.06	19.70	56.18
7.0-8.9	37	22	888	40.36	24.00	59.46
9.0-10.9	4	2	5	2.50	1.25	50.00

TS: total shrimp, PS: parasitic shrimp, PN: parasite numbers

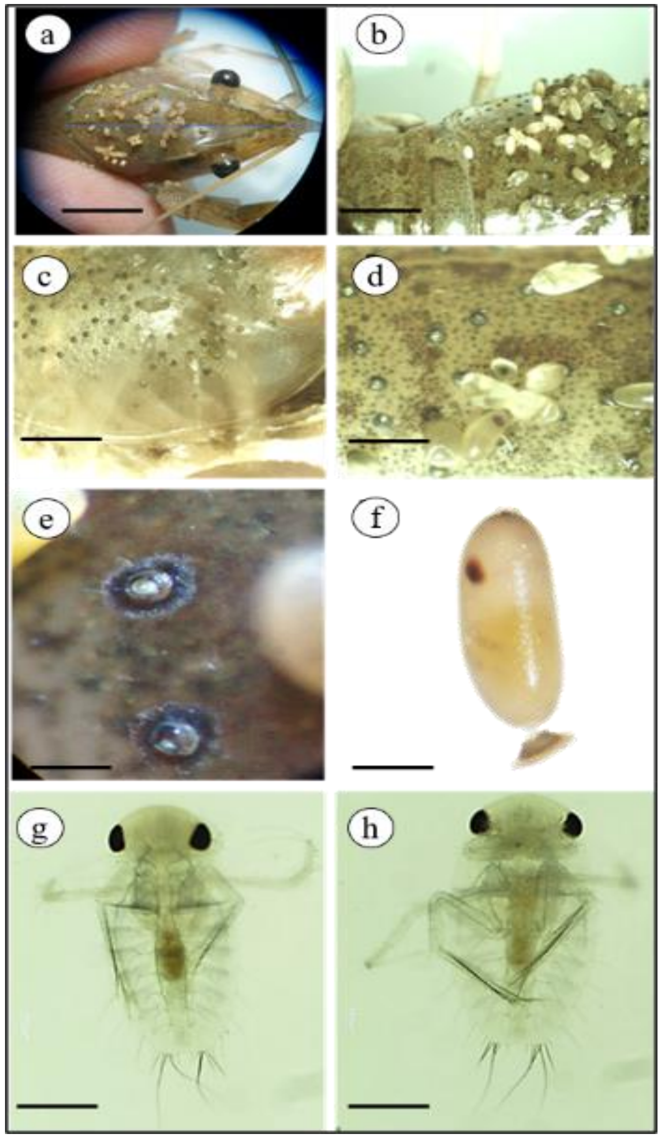

Figure 1. Infestation by *Sigara* sp. insect eggs in *Procambarus llamasi* collected in Los Rieles in Tenosique, Tabasco, Mexico. a-b View of the cephalothorax infested with eggs, c and d) Side view of the cephalothorax, e) View of the base of the insect egg, f) Egg anatomy showing peduncle and cementing base of *Sigara* sp, g) Dorsal view of *Sigara* sp nymph, recently hatched, h) Ventral view of *Sigara* sp, recently hatched.

Figure 5. Developing *Sigara* sp a) Nymph, b) Breeding of *Sigara* sp nymph in fish tank, b) *Sigara* sp. adult.

in southern California and the state of Morelos, Mexico (Smith, Sandifer and Manzi, 1979; Johnson, 1995; Vásquez *et al*., 2005; Longshaw, 2011). Regarding the infestation habitat, the eggs of *Sigara* sp. were located in the cephalothorax of *P. llamasi* and, have been reported in *M. rosenbergii* and *M. tenellum*, as well as in the first and second abdominal segments of *Macrobrachium* (Smith, Sandiger and Manzi, 1979; Johnson, 1995; Vásquez *et al.*, 2005), in some cases the crayfish could not move their eyestalks (Meyer, 1965). There are no records that this and other insects in the Corixidae family and the *Sigara* genus cause mortality in crayfish and other shrimp. The search for associations between Length and MI as well as with the AB and P indicates that no statistical relationship exists between these variables ($p < 0.05$). LT-MI (Spearman *rs* = -0.20; p=0.31); LT-AB (Spearman *rs* = -0.40; p = 0.60) and LT-P (Spearman *rs* = -0.40; p = 0.60). These similar to report Vásquez *et al*., (2005) in *Macrobrachium rosenbergii*, were no encounter relation between size and infest of egg epibiont the *Ramphocorixa* sp. and *R. acuminate.* However, the negative impact is that they are predators of larvae and juveniles of fish and crustaceans, so they can be harmful to aquaculture (Roig-Juñent, Claps and Morrone, 2014).

CONCLUSION

The eggs located on the cuticular surface of the cephalothorax of *Procambarus* (*Austrocambarus*) *llamasi* correspond to the genus *Sigara* sp. Sizes with the highest infestation indexes were 5.0-6.9 cm and 7.0-8.9 cm LT. Also, males had higher prevalence with respect to females. It was determined that the eggs on the shrimp's cuticular surface cause cuticle damage due to cementation on its shell. Eggs can also cause esthetic damage affecting marketing for human consumption and make shrimp overweight due to the egg mass. It is also suggested that recently hatched nymphs could serve as food for the different phases of crayfish growth. *P. llamasi* serves as a substrate for the eggs of *Sigara* sp. But it can also be conditioned by the environmental ecological regulations of the place, such as the type of substrate, the cover, the size of the plants, and the ecosystem.

Acknowledgments
This paper resulted from joint efforts between the Universidad Juárez Autónoma de Tabasco, the Programa de Mejoramiento del Profesorado (PROMED). The authors thank Francisco Hernandez-Hernandez for his outstanding technical assistance in preparation of material

Funding: No funds of any kind were obtained to carry out this study.

Compliance with ethical standards. The research work did not involve human subjects

Conflict of interest. The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Data availability. The data is available with the author by correspondence (h_raul_e@hotmail.com), upon request

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