ISSN Versión impresa 2218-6425 ISSN Versión Electrónica 1995-1043

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ASOCIACIÓN PERUANA DE HELMINTOLOGÍA E INVERTEBRADOS AFINES

ORIGINAL ARTICLE /ARTÍCULO ORIGINAL

OCCURRENCE OF *HETEROPRIAPULUS HETEROTYLUS* (MONOGENOIDEA: DACTYLOGYRIDAE), ECTOPARASITE OF TWO INVASIVE SAILFIN CATFISHES (SILURIFORMES: LORICARIIDAE) FROM THE SOUTHEASTERN MEXICO

OCURRENCIA DE *HETEROPRIAPULUS HETEROTYLUS* (MONOGENOIDEA: DACTYLOGYRIDAE), ECTOPARASITO DE DOS PECES GATO INVASORES (SILURIFORMES: LORICARIIDAE) EN EL SURESTE DE MEXICO

María Amparo Rodríguez-Santiago[1,2*], Mayra I. Grano-Maldonado[3,4], Enrique Ávila[5] & Samuel Gómez[2]

*[1]Catedras CONACyT, Universidad Autónoma del Carmen, Facultad de Ciencias Naturales, Centro de Investigación de Ciencias Ambientales (CICA), Av. Laguna de Términos s/n Col. Renovación 2da Sección, C.P. 24155 Ciudad del Carmen, Campeche, México. Tel. 938 93811018 EXT 180. *Current address corresponding author: marodriguez@conacyt.mx , amparoshalom@hotmail.com*
[2]Instituto de Ciencias del Mar y Limnología, Unidad Académica Mazatlán, Universidad Nacional Autónoma de México, Joel Montes Camarena s/n, 82040. Mazatlán, Sinaloa. samuelgomez@ola.icmyl.unam.mx [3]Departamento de Ecofisiología, Facultad de Ciencias del Mar, Universidad Autónoma de Sinaloa. Paseo Claussen s/n. A.P. 610. Mazatlán, Sinaloa. México.[4]CCMAR-CIMAR L.A., Centro de Ciências do Mar; Universidade do Algarve, Faro, Portugal grano_mayra@hotmail.com; mgmaldonado@ualg.pt
[5]Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Estación El Carmen, Carretera Carmen-Puerto Real km. 9.5, 24157, Ciudad del Carmen, Campeche, México. kike@ola.icmyl.unam.mx

Neotropical Helminthology, 2015, 9(1), jan-jun: 55-64.

ABSTRACT

The present study reports the presence of the dactylogyrid parasite *Heteropriapulus heterotylus* on the gills of the invasive Amazon sailfin catfishes *Pterygoplichthys pardalis and P. disjunctivus* from freshwater ecosystems in Southeastern Mexico. This represents a new host record for this parasite and a new geographical record of parasites for *P. disjunctivus* and *P. pardalis*. Furthermore, the ecological aspects of the prevalence, mean abundance and mean intensity of *H. heterotylus* were examined throughout an annual cycle. A total of 223 fish were collected, including 94 of *P. disjunctivus* and 129 of *P. pardalis*. A total of 148 (66.3%) fish were found to be infected with this dactylogyrid, 79 of *P. pardalis* and 69 of *P. disjunctivus*. *H. heterotylus* was found exclusively in the gill arches in both hosts. In both fish, the mean abundance of this parasite showed significant changes during the year. Likewise, prevalence and mean intensity showed similar values in both hosts. The present study demonstrates that parasitological studies in non-native fish can be helpful in designing control strategies for the importation of non-native fish and should be regarded as necessary before encouraging fish consumption as a control strategy in regions where these invasive fish proliferate excessively.

Keywords: Dactylogyridae – *Heteropriapulus* - invasive fish – Loricariidae – Mexico - non-native fish.

RESUMEN

El presente estudio reporta la presencia del parásito dactylogirido *Heteropriapulus heterotylus* en las branquias de peces invasivos *Pterygoplichthys pardalis* y *P. disjunctivus* en ecosistemas de agua dulce del sureste de México. Este studio representa un nuevo registro de hospederos para este parásito y un nuevo registro geográfico de parasitos en *P. disjunctivus* y *P. pardalis*. Además, se examinaron los aspectos ecológicos de la prevalencia, abundancia media e intensidad media de *H. heterotylus* a través de un ciclo anual. Se recolectaron un total de 223 peces, incluyendo 94 de *P. disjunctivus* y 129 de *P. pardalis*. Se encontró un total de 148 (66.3%) peces que estaban infectados con este monogeneo, 79 de *P. pardalis* y 69 de *P. disjunctivus*. *H. heterotylus* se registró exclusivamente en los arcos branquiales en ambos peces. En ambos peces la abundancia media de este parasito mostró cambios significativos a través del año. Asimismo, la prevalencia e intensidad media mostraron valores similares en ambos hospederos. El presente estudio demuestra que los estudios parasitológicos en peces exóticos o no nativos pueden ser útiles en el diseño de estrategias de control para la importación de estos peces y deben ser considerados como necesarios antes de fomentar el consumo de peces como una estrategia de control en las regiones donde estos peces invasores proliferan en exceso.

Palabras clave: Dactylogyridae – *Heteropriapulus* – Loricariidae – México - peces invasores - peces no nativos.

INTRODUCTION

The introduction of exotic or non native aquatic species is one of the most critical environmental risks currently facing native species, aquatic habitats and biodiversity in general (Torres-Orozco & Pérez-Hernández, 2011). This phenomenon is responsible for the extinction of 54% of the world's native aquatic fauna, 70% of fish in North America and 60% of Mexican fish (IMTA *et al*., 2007). Amazon sailfin catfishes (Siluriformes: Loricariidae) are among the exotic fish that pose the most serious threat to tropical and subtropical freshwater regions (Liang *et al*., 2005, Chavez *et al*., 2006; Nico, 2010). Native to South America, these fish have been introduced across the world by the aquarium trade and occasional escapes due to owner carelessness and have now established themselves in tropical and subtropical freshwater bodies. For example, *Pterygoplichthys pardalis* (Castelnau, 1855) and *Pterygoplichthys disjunctivus* (Weber, 1991) are reportedly destroying cages and nets and causing a decline in native, more desirable fish in Laguna de Bay, Philippines (Chavez *et al*., 2006). *P. disjunctivus* attaches to the skin of the Endangered native Florida manatee (*Trichechus manatus* ssp. *latirostris)* and feeds on their epibiota. In some instances dozens of *P. disjunctivus* and manatees appeared agitated. This interaction may be detrimental to manatee but remains unclear (Nico *et al*., 2009). *P. pardalis* damage fishing gear and gill nets in various locations of Mexico (Wakida-Kusunoki *et al*., 2007). The introduction and subsequent establishment of the amazon sailfin catfish species *P. pardalis* and *P. disjunctivus* in extraneous ecosystems has been demonstrated. Recently, these fish species were listed among the top 30 invasive species in Taiwan (Lee *et al*., 2006). They prey on and compete with native species (Hill & Lodge, 1999), spread parasites and pathogens (Torchin *et al*., 2003; Torchin & Mitchell, 2004), cause unexpected hybridization

(Gaskin & Schaal, 2002; Mallet, 2007) and economic loss to fisheries (Chavez *et al*., 2006; Nico, 2010), and may ultimately cause a decrease in local biodiversity by eliminating local species (Chapin *et al*., 2000; Dick & Platvoet, 2000).

In Mexico, the presence of Loricariids in wild environments was first reported in the mid-90s (Guzman & Barragán, 1997). In 2007, Wakida-Kusunoki *et al*. reported the presence of *P. disjunctivus* and *P. pardalis* (Actinopterygii, Loricariidae) in freshwater ecosystems of Southeastern Mexico. Currently, these species are listed as one of the top invasive species in Mexico (Mendoza-Alfaro *et al*., 2011), and they continue to spread through wild environments (rivers, streams, reservoirs and lakes) in several parts of the country, proliferating excessively. The main impact of the introduction of these fish is the competition for space and food with native species, including commercial fish species (Martínez-Palacios *et al*., 2010a, 2010b; Wakida-Kusunoki *et al*., 2011; Sandoval-Huerta *et al*., 2012). Despite the threat *P. disjunctivus* and *P. pardalis* pose, there is currently little information available about their parasitic fauna (Mendoza-Franco *et al*., 2012). Some species of monogenea, such as *Gyrodactylus cichlidarum*, *Dactylogyrus vastator* (Caspeta *et al*., 2009) and *G. anguillae* (Grano-Maldonado *et al*., 2011), are regarded as high risk in aquaculture because they are associated with native fish mortalities. In this study, we report for the first time the presence of *H. heterotylus*, a dactylogyrid ectoparasite specific to the gills of *P. pardalis* and *P. disjunctivus*, in the freshwater ecosystems of Southeastern Mexico and compare the abundance and prevalence of the monogenoidean in these coexisting congener hosts and determine whether the inter-annual variability in the abundance of this parasite in each fish is related to the water temperature and/or the host size and weight.

MATERIALS AND METHODS

In 2012, invasive sailfin catfish specimens were captured from freshwater ecosystems in Campeche in Southeastern Mexico. Fish specimens were sacrificed by decapitation to ensure a fast dead, which is according to Mexican laws (NOM-033-ZOO-1995). The fishing area was located in the Palizada River within the Flora and Fauna Protection Area Laguna de Terminos (la Rivera: 18°20'01”N, 91°41'55”W). The fish were caught (bimonthly) between February and December 2012 with fishing nets (mesh = 76 mm) and transported alive (individually) to the laboratory in plastic containers. At each sampling site, the bottom water temperature was recorded using a salinity/conductivity/temperature meter (YSI-30, Ohio 45387 USA). For each fish, the results of the parasitological examination as well as the fish's total length (cm), standard length (cm) and weight (g) was recorded. The parasitological survey included a revision of external organs: eyes, fins, skin, and gill cavities. The gill cavities were individually separated and subsequently checked by compression between two 10 x 10 x 1.0 cm glasses (Guzman-Cornejo *et al.* 2012). The parasites were preserved in individual labeled vials with 96% alcohol until definitive identification. The monogenoideans were individually mounted on a slide, and the proteolytic digestion method was used to release the surrounding tissue of the haptoral sclerites (see detailed method in Harris & Cable, 2000). Complete specimens were mounted in Gray and Wess media to clear the tissue and visualize the sclerites and male copulatory organ. The specimens were examined under an Olympus BX-51 microscope. The identification of the dactylogyrid species was based on the characteristics of the sclerotized hard parts of the haptor and the male copulatory organ. The body morphology and sclerotized organs of each individual were photographed using a digital camera (Olympus C-5050 Zoom) attached to a microscope. These ectoparasites were stained with Gomori's trichrome or Mayer's carmine to determine the internal features as described by Kritsky *et al.* (1986). The parasites were identified using keys proposed by Jogunoori *et al*. (2004) and Kritsky (2007). The parasitological material was deposited in the National Helminths Collection of the Institute of Biology of the National Autonomous University of Mexico (CNHE-IBUNAM). The prevalence (%), mean intensity (total number of parasites of a particular species found in a sample/the number of hosts infected with that parasite) and mean abundance (number of individuals/host) of the parasites were determined as described by Bush *et al*. (1997). To assess significant variations in the mean abundance of parasites over seasons, a Kruskal-Wallis non-parametric analysis of variance was performed, which was followed by Tukey HSD post hoc test (Steel & Torrie, 1986). To assess significant differences between mean abundance and mean intensity of parasites between both fish hosts Mann-Whitney U test was used. Spearman rank correlations were used to assess relationships between the abundance and intensity of parasites and host size and weight, fish condition and bottom water temperature. The Fulton's Condition Factor (KLP) was calculated as K = [W/TL3] 10,000, where: W = weight (g) and TL = total length (mm), to describe the physiological condition of each individual fish (Ricker 1975).

RESULTS

From February to December 2012, a total of 223 fish specimens of *P. pardalis* and *P. disjunctivus* were collected and examined. During the study period, the average water temperature ranged from 24ºC (in February) to 30.8ºC (in August). Of the total fish examined, only 148 (66.3%) were infected with ectoparasites.

A total number of 604 parasite individuals were found in *P. pardalis* and *P. disjunctivus*. The parasite species found was identified as *Heteropriapulus heterotylus* (*P. pardalis* n = 408, *P. disjunctivus* n = 196). This monogenean was found specifically in the gill arches.

The mean abundance of *H. heterotylus* in *P. pardalis* and *P. disjunctivus* showed significant variations over the course of the study (ANOVA, $P < 0.01$). The mean abundance ± SE of *H. heterotylus* was of 1.56 ± 0.58 ind.host^{-1} in *P. pardalis* and 1.20 ± 0.20 ind.host^{-1} in *P. disjunctivus*. In both hosts, this parasite was relatively high between June and September (Table 1) and absent in December for *P. disjunctivus*.

The prevalence of *H. heterotylus* was of 61.6% in *P. pardalis* and 64.6% in *P. disjunctivus*. This population descriptor had a peaked twice between April and November (Table 1). The mean intensity of *H. heterotylus* was higher in *P. pardalis* (3.7 ± 1.2) than in *P. disjunctivus* (2.4 ± 0.6), although this difference was not statistically significant (Mann-Whitney U test, $P > 0.05$). Moreover, in *P. pardalis*, the mean intensity of infection of *H. heterotylus* was higher in September (10.7 ± 2.3) and lower in January (1.0 ± 0.0). In *P. disjunctivus*, the mean intensity was also higher in September (4.6 ± 0.4) and lower in December (0.0 ± 0.0).

For *P. pardalis,* significant relationships were found between the water temperature and both the mean intensity and the mean abundance of *H. heterotylus* (mean intensity-temperature, $r_s = 0.77$, $P < 0.05$; mean abundance-temperature, $r_s = 0.85$, $P < 0.01$). In *P. disjunctivus*, a significant relationship was found between the mean abundance of *H. heterotylus* and water temperature ($r_s = 0.77$, $P < 0.05$). Moreover, no significant relationships

Table 1. Prevalence (P%), mean abundance (MA) and mean intensity (MI) of *H. heterotylus* in the invasive Amazon sailfin catfishes *P. pardalis* and *P. disjunctivus* from a freshwater ecosystem of Southeastern Mexico (n = 223). Total number of host individuals and size range in cm (H), total number of parasites (PT).

Parasites		H	PT	P (%)	MA	MI
Pterigoplichthys pardalis						
	Jan	16 (19-40)	5	31.2	0.16 ± 0.70	1.0 ± 0.0
	Feb	21 (27-39)	37	69.1	1.23 ± 0.44	2.92 + 0.3
	Mar	13 (24-32)	8	24	0.47 ± 0.20	1.60 ± 0.2
	Apr	16 (26-49)	39	75	1.3 ± 0.37	3.25 ± 0.6
	Jun	14 (26-39)	91	93	3.5 ± 0.85	6.50 ± 1.0
	Sep	17 (17-38)	191	71	4.42 ± 0.96	10.7 ± 2.3
	Nov	22 (24-43)	31	95.4	0.96 ± 0.29	2.50 ± 0.5
	Dec	10 (27-40)	6	40	0.46 ± 0.22	1.50 ± 0.2
Pterigoplichthys disjuntivus						
	Jan	14 (26-39)	5	36	0.33 ± 0.17	1.0 ± 0.0
	Feb	9 (27-40)	23	89	1.58 ± 0.81	2.86 ± 0.6
	Mar	3 (25-29)	5	56	0.3 ± 0.22	1.67 ± 0.5
	Apr	14 (19-40)	47	100	1.74 ± 0.40	3.36 ± 0.4
	Jun	12 (25-41)	45	83	3 ± 1.54	4.50 ± 0.6
	Sep	15 (25-37)	57	53	2.38 ± 0.40	4.57 ± 0.4
	Nov	24 (18-48)	14	100	0.3 ± 0.11	1.43 ± 0.2
	Dec	3 (31-43)	0	0	0.0 ± 0.0	0.0 ± 0.0

were found between the host size, weight and condition factor of the hosts and the community descriptors of parasites (mean abundance, prevalence and mean intensity).

DISCUSSION

The present study provides new data on the prevalence and abundance of monogenean *H. heterotylus*, (Monogenoidea: Dactylogyridae) parasitizing on two invasive Amazon sailfin catfishes (Siluriformes, Loricariidae) in Southeastern Mexico. This monogenean *H. heterotylus* is very likely specific to loricariids of South American origin, and is probably native to America itself, however, so far it has been reported only from Asia (India, China, Japan) and Mexico, into which loricariids have been introduced (Jogunoori *et al.*, 2004; Li & Huang, 2012; Mendoza-Franco *et al.*, 2012; and in the present study). Wild loricariids from South America need to be examined for the presence of this parasite. The fact to find the same parasite species *H. heterotylus* in the present study, in the two species of *Pterygoplichthys* may suggests a possible specificity by these host species that also share the same environment. If so, *H. heterotylus* could be regarded as an introduced species of parasite as the fish host. However, the possible transfer of this parasite to native fish species remains unknown. This fact has widely documented in parasites of ornamental fishes (with wider host specificity), which may acquire new host species in a newly invaded environment (King & Cable, 2007).

This report is the first record of the species *Pterygoplichthys* by the genus *Heteropriapulus*. The fish *P. pardalis* and *P. disjunctivus* are natives of the inland waters of Central and South America, which were introduced to Mexico through aquarium trade (Wakida-Kusunoki, 2008). This represents a new host record for this parasite and a new geographical record for *P. disjunctivus* and *P. pardalis*. The presence of the monogenean *H. heterotylus* was highlighted, due of its relative high abundance and prevalence in both fish host species during the study. The species of this genus have been reported before only in exotic fishes, in aquaria, rearing facilities or introduced into local lakes and streams, have frequently been found to host dactylogyrids that occur on the respective hosts in their native habitats (Jogunoori *et al.*, 2004, Wakida-Kusunoki, 2008). The significant positive relationships found in both hosts between the mean abundance of parasites and the water temperature indicated that these parasites, particularly *H. heterotylus,* showed a marked seasonality, likely due to environmental changes. These findings agree with other studies that suggest that interannual variability in the abundance of parasites in a host may be due to seasonal changes in water temperature (Chubb, 1977; Scott & Nokes, 1984; Koskivaara *et al.*, 1991). For example, a parasitological study on the fish *Puntius* spp. (from India) revealed that abundance of monogeneans showed an increasing or decreasing trend with rise and fall in temperature, in general, although some species were more abundant during the monsoon and winter seasons (Shrivastava *et al.*, 2012). It was also suggested that increase of parasites during monsoon can be explained by the synchronization of parasite life cycle with the beginning of host reproduction. Also, during monsoon conditions there is a low amount of dissolved oxygen in water, which many fish are forced to come to the surface to breathe, increasing the chances of contact with free-swimming oncomiracidia hatched from floating eggs (Shrivastava *et al.*, 2012). The higher abundance of parasites recorded in this study during June-September (rainy season), could also be related to a more stressful conditions for the hosts and therefore be more vulnerable to parasites, as the water turbidity increases considerably during this period. In a previous study conducted within this same region, Wakida-Kusunoki & Amador del Ángel (2011) found that the abundance of these loricariid species was relatively higher from June to September, which was positively correlated with the gonadosomatic index and the river water level. In this sense, it is also possible that the season of higher abundance of parasites is related to the reproductive stage of the host, since it is well known that some fish species are more susceptible to parasite infection in periods of higher reproductive investment (Simková *et al*,. 2005).

Surprisingly, the same situation has been documented for *H. heterotylus*, which has been found in other exotic members of the family Loricariidae (*Hypostomus* sp.; Jogunoori *et al.*, 2004, Kritsky, 2007; Wu *et al.*, 2011). These findings agree with Poulin *et al.* (2011) who suggested that sympatric or phylogenetically related hosts with similar ecologies should have similar parasite communities. The parasite *H. heterotylus* is very likely specific to loricariids and is probably native to South America; however, its distribution has expanded to Asia, including India, China, and Japan (Nitta & Nagasawa, 2013).

The identification of the same parasite, *H. heterotylus*, in both *P. pardalis* and *P. disjunctivus* suggests a possible common specificity by these host species that also share the same environment. If so, *H. heterotylus* could be regarded as an introduced parasite species. However, the transfer of this parasite to native fish species has not yet been demonstrated. Certainly, the transfer of parasites of ornamental species with wider host specificity to native species in newly invaded environments has been widely documented (King & Cable, 2007). In Mexico, some species of parasites such as *G. cichlidarum* and *D. vastator* have been regarded as high risk in aquaculture because they are associated with fish mortalities (Caspeta *et al.*, 2009). Therefore, we strongly recommend assessing the possible routes of propagation of these fish species throughout the country as well as analyzing other coexisting fish species to determine whether these parasites have been transferred to other species.

In summary, this study documents the parasite community of the Amazon sailfin catfish species *P. pardalis* and *P. disjunctivus* which are known to be highly invasive in many regions worldwide. In general, the parasite species richness in these invasive fishes was relatively low. The monogenean *H. heterotylus* are species which shared these fish hosts, which seems to be specific of loricariids as also occur with its congener *H. heterotylus* found in other loricariid species from India (Jogunoori *et al.*, 2004). Also, interannual variations in the overall mean abundance and intensity of monogenean were detected, which were positively related to the water temperature.

Finally, the present work shows that parasitological studies in exotic fish can also be useful to design control strategies, and should be regarded as necessary before encourage its consumption as a control strategy in those regions where these invasive fish species proliferate excessively. It is strongly recommended to conduct further studies about the possible incidence of these parasites on native species, which share the same environment with these loricariid species.

ACKNOWLEDGEMENTS

We would like to thank to Erik Hoberg and Patricia Pilitt from the USNPC for the pictures of type and voucher specimens of *Heterotylus heterotylus* from India. We would like to thank to Alvarez-Guillén H, Reda-Deara A. and Gómez-Ponce A. for their help in the field samplings. To Gerardo Perez-Ponce de Leon by providing us materials and equipment in the laboratory of Helmintología, UNAM. MARS and MIGM thanks to CONACyT (Consejo Nacional de Ciencia y Tecnología) and the Instituto de Ciencias del Mar y Limnologia Unidad Academica Mazatlan by the Postdoctoral Fellow and support.

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Received, March 6, 2015
Accepted, April 18, 2015