Parasites of two coexisting invasive sailfin catfishes (Siluriformes: Loricariidae) in a tropical region of Mexico

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Currently many species of Amazon sailfin catfishes (Loricariidae) have been introduced to wild environments outside their native range. There is, however, little knowledge about their role as vectors of parasites that can infect native fish or even humans through its consumption. The aim of the present study was to determine the parasitic fauna of the invasive sailfin catfish species *Pterygoplichthys pardalis* (leopard pleco) and *P. disjunctivus* (vermiculated pleco) from freshwater systems in the southeast of Mexico. Four ectoparasite species were found in *P. pardalis* (1 protozoan: *Ichthyophthirius multifiliis*; 2 monogeneans: *Urocleidoides vaginoclastrum* and *Heteropriapulus heterotylus*; 1 digenean: *Clinostomum* sp.), and only one in *Heteropriapulus disjunctivus* (*H. heterotylus*). No endoparasites were found. *Ichthyophthirius multifiliis*, *U. vaginoclastrum* and *Clinostomum* sp., were considered as rare species (prevalence <5%) since they were found in a single individual of *P. pardalis*. *H. heterotylus* was the only species shared among both host species and it occurs throughout the year. This monogenean species represents 96% of total parasites recorded in *P. pardalis* and 100% in *P. disjunctivus.* Monthly values of prevalence, intensity and abundance of *H. heterotylus* in both host species showed important intra-annual variations, but not differ significantly between both hosts.

Actualmente muchas especies de peces conocidos como plecos o peces diablo (Loricariidae) han sido introducidas en ambientes silvestres fuera de su área de distribución natural. Sin embargo, hay poco conocimiento acerca de su papel como vectores de parásitos que puedan infectar a los peces nativos o incluso los seres humanos a través de su consumo. El objetivo del presente estudio fue determinar la fauna parasitaria de las especies de plecos *Pterygoplichthys pardalis* (pleco leopardo) y *P. disjunctivus* (pleco vermiculado) en sistemas de agua dulce del sureste de México. Fueron encontradas cuatro especies de ectoparásitos en *P. pardalis* (1 protozoario: *Ichthyophthirius multifiliis*; 2 monogeneos: *Urocleidoides vaginoclastrum* y *Heteropriapulus heterotylus*; 1 digeneo: *Clinostomum* sp.) y una en *P. disjunctivus* (*H. heterotylus*). No se encontraron endoparásitos. *Ichthyophthirius multifiliis, U. vaginoclastrum* y *Clinostomum* sp. fueron consideradas como especies raras (prevalencia <5%), ya que estuvieron en un solo individuo de *P. pardalis*. *Heteropriapulus heterotylus* fue la única especie compartida entre ambas especies de peces y que estuvo presente durante todo el año, y representó el 96% del total de parásitos registrados en *P. pardalis* y el 100% en *P. disjunctivus.* La prevalencia, intensidad media y abundancia media de *H. heterotylus* mostraron importantes variaciones intra-anuales en ambas especies de peces, pero no difirió significativamente entre ambos hospederos.

Key Words: Exotic fish, Parasitic fauna, Campeche, Leopard pleco, Vermiculated pleco.

Introduction

Introduction of non-native fish species threatens native biodiversity and the ecosystem function of aquatic communities globally (Kelly *et al.,* 2009; Cucherousset & Olden, 2011). Many non-native fish species may become predators, competitors (Hill & Lodge, 1999), or even spread parasites or pathogens to native species (Torchin *et al*., 2003; Torchin & Mitchell, 2004). The latter has been the subject of concern for many decades, since it is well known that parasites transmitted by non-native species can cause severe impacts on native species (Bakke *et al.,* 1990; Poulin *et al.,* 2011).

Amazon sailfin catfishes, also known as "armored catfishes", are endemic to freshwater ecosystems of South and Central America and some species have recently been reported in wild environments outside their native range (*e.g.* North America: Fuller *et al.*, 1999; Capps, 2008; Gibbs *et al.,* 2008; Pound *et al.,* 2011; Puerto Rico: Bunkled-Williams *et al.,* 1994; México: Guzmán & Barragán, 1997; Wakida-

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Kusunoki *et al.,* 2007; Wakida-Kusunoki & Amador-del Ángel, 2011; Taiwan: Liang *et al*., 2005, 2006; Philippines: Chavez *et al.,* 2006; Turkey: Özdilek, 2007; Poland: Keszka *et al.,* 2008; Vietnam: Levin *et al*., 2008; Java and Australia: Capps, 2008; Bangladesh: Hossain *et al*., 2008; Italy: Piazzini *et al*., 2010; Serbia: Simonović *et al.,* 2010 and Tailand: Chaichana & Jongphadungkiet, 2012). It has been suggested that in most of those recipient areas recorded so far, aquarists were assigned responsibility for releasing the catfishes into natural ecosystems and the their subsequent establishment (Simonović *et al., 2010*).

The first report of a wild sailfin catfish in Mexico was made by Guzmán & Barragán (1997) in the Mezcala River, Guerrero. More recently, Wakida-Kusunoki *et al.* (2007) reported the presence of *Pterygoplichthys disjunctivus* (Weber, 1991) and *P. pardalis* (Castelnau, 1855) in freshwater ecosystems of southeastern Mexico. Currently, these species are listed as one of the most invasive species in Mexico (Mendoza-Alfaro *et al.,* 2011) and continue spreading and proliferating massively throughout rivers, streams, reservoirs and lakes of several parts of the country. The main problem caused by these species is the competition for space and food with native fish species, including those commercially important (Martínez-Palacios *et al.,* 2009; Sandoval-Huerta *et al.,* 2012). However, nowadays there is little information available about their parasitic fauna (Mendoza-Franco *et al.,* 2012) and the role of these parasite species as potential pathogens to native fish species or humans (Pound *et al.,* 2011).

Therefore, the aims of this study were (1) to determine the parasitic fauna of the invasive sailfin catfish species: *P. pardalis* and *P. disjunctivus* from riverine systems of southeastern Mexico, (2) to compare the population parameters of infection (prevalence, intensity and abundance) among these coexisting fish hosts and (3) to determine whether the intra-annual variability in the prevalence, mean intensity and mean abundance of parasites in each fish species is related to seasonal changes in water temperature and/or to the host size and weight.

Material and Methods

Study area and sampling method. Specimens of the leopard pleco *Pterygoplichthys pardalis* and the vermiculated pleco *P. disjunctivus* were collected from two localities in the Palizada River (Santa Gertrudis: 18˚20'04.7"N, 91˚41'56.1"W and La Rivera: 18˚20'01"N, 91˚41'55"W) within the Natural Protected Area Laguna de Terminos, Campeche, Mexico.

Fish were caught using a casting net (mesh = 76 mm) at depths between 0.7 m to 2.0 m depth (from January to December 2012) and transported alive (individually) to the laboratory in plastic containers. At each sampling site, the bottom water temperature was recorded by using a multiparameter sensor (YSI-30 Salinity, Conductivity Temperature, Ohio 45387 USA). During the study period (January-December 2012) the average water temperature ranged from 24ºC (in February) to 30.8ºC (in August) (Fig.

1b). Fish were sacrificed by decapitation to ensure a fast dead, which is according to Mexican laws (NOM-033-ZOO-1995). The total length (cm) and weight (g) were recorded for each fish. The parasitological survey included the revision of external (eyes, fins, skin, and gill cavities) and internal organs (intestinal caeca, coelomic cavity, stomach, intestine, spleen, gallbladder, liver, kidneys, and heart) and muscle. Internal organs were individually separated and subsequently examined by compression between two 10 x 10 x 1.0 cm flat glasses (Guzmán-Cornejo *et al.,* 2012). Parasites were isolated and preserved in labelled vials with 96% alcohol.

Identification of monogeneans was based on the characteristics of the sclerotized hard parts of haptor and male copulatory organ. Haptors were subjected to a partial digestion using a proteinase K- base method following Harris *et al.* (1999) in order to study haptoral sclerites. Trematodes were stained with Gomori's trichrome or Mayer's carmine to determine the internal features. Skin and gills smears were made in search of protozoans, which were impregnated with silver nitrate (Martorelli *et al.,* 2008). Voucher specimens of helminths were deposited at the Colección Nacional de Helmintos, Instituto de Biología, Universidad Nacional Autónoma de México (Mexico).

Data analyses. Prevalence, intensity and abundance were determined as recommended by Bush *et al.* (1997). Assumptions of normality and homocedasticity in each variable were previously tested by Kolmogorov-Smirnov's and Bartlett's tests, respectively (Sokal & Rohlf, 1995). To assess significant variations in the overall abundance of parasites over the year, a non-parametric Analysis of Variance of Kruskal-Wallis was used (Steel & Torrie, 1986). Chi-square tests were used to compare the prevalence and intensity between both host species (Rózsa *et al.,* 2000). Moreover, a Mann-Whitney U-test was used to assess significant differences in the abundance of parasites between hosts (Zar, 1999). Spearman rank correlation analyses were used to assess significant relationships between the population descriptors of parasites (prevalence, intensity and abundance) and fish host size and weight, and water temperature. For these analyzes, the average monthly values were used. *P*-values less than 0.05 were considered significant.

Results

Species composition and population descriptors of parasites. A total of 223 specimens of the two species of sailfin catfish were collected: 94 vermiculated pleco (total length 18-48 cm, weight 94-506 g) and 129 leopard pleco (total length 17-49 cm, weight 75-512 g). From the total fish individuals, 148 (66%; 79 *Pterygoplichthys pardalis* and 69 *Pterygoplichthys disjunctivus*) were infected with at least one parasite species.

Four parasitic species were found in *P. pardalis* (2 monogeneans: *Urocleidoides vaginoclastrum* Jogunoori, Kritsky and Venkatanarasaiah 2004 and *Heteropriapulus*

heterotylus Jogunoori, Kritsky & Venkatanarasaiah, 2004, 1 protozoan: *Ichthyophthirius multifiliis* Fouquet 1876, and 1 trematode: *Clinostomum* sp.); whereas only one occurred in *P. disjunctivus* (*Heteropriapulus heterotylus*). *H. heterotylus*, *U. vaginoclastrum* and *I. multifiliis* were found on gill arches and the trematode *Clinostomum* sp. was found encysted on the base of rays of caudal fin. In both host fish species, internal organs did not present parasites. In *P. pardalis,* 436 individual parasites were collected of which 408 (96%) were *Heteropriapulus heterotylus*; 23 (3.5%) *I. multifiliis*; 3 (0.5%) *U. vaginoclastrum* and 2 (0.3%) *Clinostomum* sp. On the other hand, 196 (100%) individuals of *H. heterotylus* occurred in *P. disjunctivus* (Table 1).

In both sailfin catfish species, the monogenean *H. heterotylus* exhibited a relatively higher prevalence (monthly prevalence values ranged from 23.8%-95.5% in *P. pardalis* and 100% in *P. disjunctivus*) followed by *U. vaginoclastrum* (4.7%), *Clinostomum* sp. (4.7%) and *I. multifiliis* (4.5%). Prevalence values of *H. heterotylus* did not vary significantly (Chi square test, $p > 0.05$) between samples of *P. pardalis* and *P. disjunctivus.*

Heteropriapulus heterotylus showed also the highest mean intensity ($\pm$ SE) (3.26 $\pm$ 1.44), and did not show a significant difference (Chi square test, $p > 0.05$) between *P. pardalis* (1.0 $\pm$ 0.0–8.9 $\pm$ 1.5 in) and *P. disjunctivus* (1.0 $\pm$ 0.0–4.6 $\pm$ 0.4). *H. heterotylus* was the most abundant parasite (until 4.65 $\pm$ 1.01 ind.host^{-1} in *P. pardalis* and 4.5 $\pm$ 0.39 ind.host^{-1} in *P. disjunctivus*). The mean abundance of the monogenean (*H. heterotylus*) was not significantly different (Mann Whitney U-test, $p > 0.05$) between both sailfin catfish species.

Temporal changes in prevalence, intensity and abundance were analyzed only in the monogenean *H. heterotylus* since this was the only species present during the whole year in both fish species (Fig. 1). In *P. pardalis*, the highest proportion of hosts infected with this parasite was recorded in June (93%) and the lowest in March (24%), where as in *P. disjunctivus* the highest values were in April and November (100%) and did not show any parasites in December (Fig. 1a). Moreover, in *P. pardalis*, the intensity of infection by this monogenean was relatively higher between June and September (6.5 $\pm$ 1.0 and 8.9 $\pm$ 1.5, respectively) and lower in January (1.0 $\pm$ 0.0).

In *P. disjunctivus*, the intensity was also higher between June and September (4.5 $\pm$ 0.7 and 4.6 $\pm$ 0.4, respectively) (Fig. 1b). Similarly, the abundance of *H. heterotylus* is higher between June and September (3.5 $\pm$ 0.9 ind.host^{-1} and 4.7 $\pm$ 1.0 ind. host^{-1}) and the lowest in January (0.2 $\pm$ 0.1 ind.host^{-1}) (Fig. 1c).
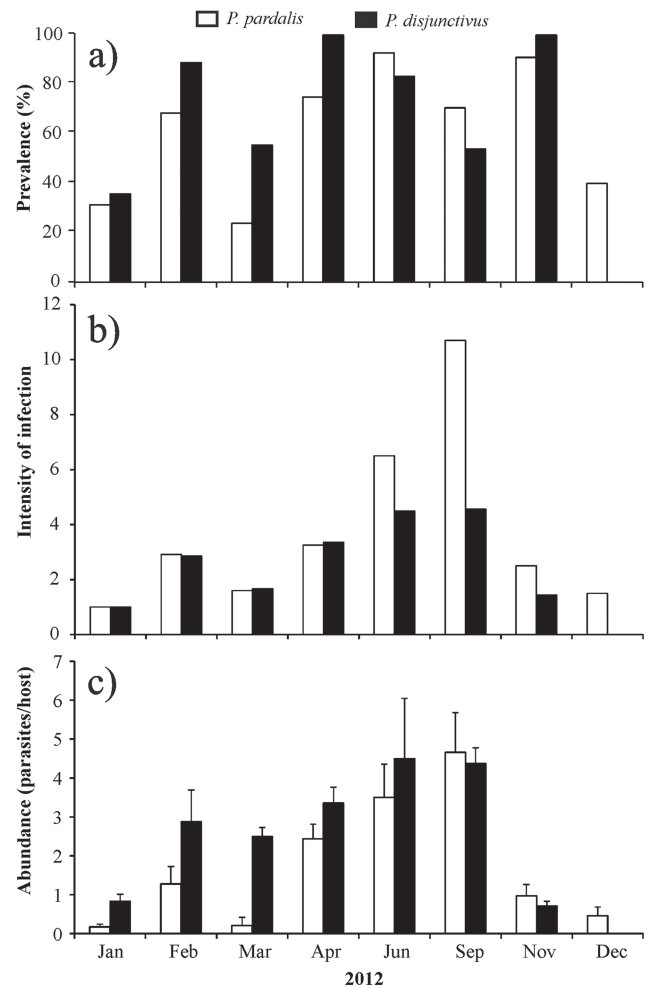

Fig.1. Intra-annual variations in: a) prevalence, b) intensity of infection and c) abundance of parasites in the invasive loricarids *Pterygoplichthys disjunctivus* and *P. pardalis* from a freshwater ecosystem of southeastern Mexico.

Table 1. Infection parameters of the parasite from *Pterygoplichthys pardalis* and *P. disjunctivus*.

Parasites	Site of infection	Prevalence (%)	Mean abundance	Mean intensity
Pterygoplichthys pardalis				
Protozoa				
Ichthyophyhirius multifilis	Gill	1	0.2 $\pm$ 0.2	2.87 $\pm$ 3.07
Monogenea				
Heteropriapulus heterotylus	Gill	62	1.59 $\pm$ 1.20	3.26 $\pm$ 1.44
Urocleidoides vaginoclastrum	Gill	0.02	0.02 $\pm$ 0.02	0.40 $\pm$ 0.42
Trematoda				
Clinostomum sp.	Caudal fin	1	0.01 $\pm$ 0.01	0.26 $\pm$ 0.28
Pterygoplichthys disjunctivus				
Monogenea				
Heteropriapulus heterotylus	Gill	65	1.20 $\pm$ 0.42	2.16 $\pm$ 0.69

Likewise, it was found that the abundance of this parasite in *P. pardalis* and *P. disjunctivus* showed significant variations (Kruskal-Wallis ANOVA, *p*< 0.01) over the year and was positively correlated with water temperature (r_s = 0.89, *p*< 0.01 and r_s = 0.79, *p*< 0.05, respectively), which ranged from 24°C (in February) to 30.8°C (in August). A significant correlation was also found between the intensity of *H. heterotylus* (in both fish hosts) and water temperature (*P. pardalis*, r_s = 0.91, *p*< 0.01; *P. disjunctivus*, r_s = 0.89, *p*< 0.01). Moreover, no significant correlations were found between the host size, weight, and infection parameters (abundance, prevalence and intensity).

Discussion

There was scarce information about the parasitic fauna in the senon-native fish species, especially in the leopard pleco and the vermiculated pleco around the world. Only two parasitological reports have been published in *Pterygoplichthys pardalis* from its native range (Central Amazonia, Brazil), where a total of 6 helminth species were found: *Megacoelium spinicavum* (Thatcher & Varella, 1981), *Austrodiplostomum compactum, Diplostomum* sp., *Gorytocephalus* sp., *Heteropriapulus* sp. and *Unilatus* sp. (Porto *et al.,* 2012). Whereas outside its native range only *Heteropriapulus* sp. has been found (Mendoza-Franco *et al.,* 2012). In *Pterygoplichthys disjunctivus*, there is no information about its parasitic fauna in its native range. However, recent studies conducted outside its native range have documented the presence of the dactylogyrid monogenean *H. heterotylus* (Jogunoori, Kritsky & Venkatanarasaiah, 2004) and the parasitic copepod *Neoergasilus japonicus* (Harada, 1930) in Okinawa, Japan (Nagasawa & Uyeno, 2012; Nitta & Nagasawa, 2013).

Out of the four species found in this study for *P. pardalis*, three of them (*I. multifiliis, U. vaginoclastrum* and *Clinostomum* sp.) had not been previously recorded, although their presence appears to be occasional, as they were found on a single individual. These parasites were likely acquired from native host species (Jogunoori *et al.,* 2004).

Another interesting result of this study was the absence of gastrointestinal parasites in both invasive fish, which is likely because these introduced species have not yet acquired a parasitic fauna that could contribute to regulating their population growth in the invaded environment (Anderson, 1978). In this regard, other studies have also suggested that the low diversity of parasite species recorded in sailfin catfish species could be related to their distinctive, very thick shell that is filled with tiny thorns, which could be a barrier for some parasite species (Whittington *et al.,* 2000). Apparently, the small number of years that these fish have been present in the new geographical range along with its feeding habits and behaviour (bentic algae/ weeds) seems to be a more probable explanation.

In the life cycle of trematodes of genus *Clinostomum*, their eggs are dispersed in the feces of fish-eating birds that usually are the definitive host. The egg gives rise to a miracidial covered with cilia that swim freely and infects a snail, resulting in a sporocyst that migrates to the intestine or liver of the host snail. Subsequently, through the food web this sporocyst is transmitted to a fish where it becomes metacercaria. The cycle is completed when a fish-eaten bird eats the fish (Olsen, 1986). In monogeneans, most species are oviparous. The ciliated larva that arises from the egg can swim and infect the only host of their life cycle. Only a few monogeneans are viviparous, among which highlight those belonging to the genus *Gyrodactylus*, in which until three generations can occur in a single host individual (Bakke *et al.,* 2007). It is also known that this type of ectoparasites can be transmitted, even without direct contact whit an infected host, and some are so specific that when the host dies they are able to find a new host of the same species (Cable & Harris, 2002). Regarding *I. multifiliis*, free-swimming theront stage parasites invade the skin and gills of host fish and rapidly transform into feeding trophonts within the epidermis (Cassidy-Hanley *et al.,* 2011). Mature trophonts exit the host tissue, giving rise to the tomont stage. Then, tomonts attach to an inert support, encyst within a gelatinous capsule, and rapidly divide to give rise to 100-1000 new infective theronts within 18-24 h at 22°C (Cassidy-Hanley *et al.,* 2011).

In this study, *H. heterotylus* was the most important parasite (in terms of prevalence and abundance) and the only one shared between both fish hosts. This parasite seems to be specific of loricarids (*e.g.* Nagasawa & Uyeno, 2012; Nitta & Nagasawa, 2013). The fact of not having found significant differences between them in the prevalence, intensity and abundance of this parasite between both hosts could be because these fish species share the same habitat, have similar behavior and food habits (Torchin *et al.,* 2001).

Moreover, our data of prevalence, intensity and abundance of this monogenean (in both hosts) were consistent to those recorded in the species of *Heteropriapulus* recorded in *P. pardalis* by Porto *et al.* (2012). In the case that these species of *Heteropriapulus* were the same, our results could indicate that the population characteristics of this parasite have been maintained, even outside its native range. Although to demonstrate it molecular studies are required.

On the other hand, monthly data of prevalence, intensity and abundance of this monogenean showed a similar temporal variability in both invasive fish. Also, a significant correlation was found (in both hosts) between the intensity and abundance of *H. heterotylus* and the water temperature, suggesting that intra-annual variability of this parasite is likely influenced by this environmental parameter, as has been documented in other parasite species (Chubb, 1977; Scott & Nokes, 1984; Khidr, 2012). For example, a study conducted on *Puntius* spp. (Cyprinidae) from India revealed that abundance of monogeneans

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showed an increasing or decreasing trend with the rise and fall in temperature in general, although some monogenean species were more abundant during the monsoon and winter seasons (Shrivastava *et al.,* 2012). Similarly, a previous study conducted within our study area revealed that the abundance of these loricariid species was higher from June to September and was positively correlated with the gonadosomatic index of these fish (Wakida-Kusunoki & Amador-del Ángel, 2011). Therefore, it could be possible that the season of higher abundance of parasites is also 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 (Šimková *et al.,* 2005).

Most parasite species recorded in this study have not been documented to be pathogenic to humans, although it is known that a species of *Clinostomum* (*C. complanatum*) has been found to infect humans (two in Korea and one in Japan) (Witenberg, 1944). This parasite was transmitted by eating raw or undercooked fish, which is linked to cultural factors of certain human populations (Park *et al.,* 2009), and was found attached to the human mucous layer of larynx and pharynx. However, although the prevalence of these trematodes was very low in these hosts (*P. pardalis* and *P. disjunctivus*), it was not recommended to consume undercooked meat fish.

In summary, this study documents the parasite composition 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 was relatively poor (three species in *P. pardalis* and one in *P. disjunctivus*). Through this study, *P. pardalis* is assigned as new host for *Clinostomum* sp., *I. multifiliis*, *U. vaginoclastrum* and *H. heterotylus* and the study area as a new geographic location for the four species of parasites.

Finally, this research highlights the importance of conducting samplings in different seasons of the year in order to make a better characterization of the parasite fauna and to determine the environmental factors influencing its population dynamics.

Acknowledgements

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