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Metazoan parasites of white mullet *Mugil curema* Valenciennes, 1836 (Perciformes, Mugilidae) from the wetlands of Pantanos de Villa, Lima, Peru

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Abstract. The present study aimed to evaluate the community of metazoan parasites of the white mullet *Mugil curema* Valenciennes, 1836, in Pantanos de Villa's wetlands Lima, Peru. Twenty-five specimens of *M. curema* were collected in September and October 2012. The fish presented average weight of 947.7 ± 513 g and average length of 30.2 ± 5.84 cm. Parasites were catalogued and evaluated using standard parasitological protocols. A total of 323 metazoan parasites were collected during the whole sampling, with mean total abundance of 12.92 specimens per fish. All the white mullets were parasitized. It was observed infection with one parasite species in ten fish (40%), two parasites species in 14 fish (56%), and three parasites species in one fish (4%). The three parasites found were metacercariae of *Ascocotyle* (*Phagicola*) *longa* Ransom, 1920 (Trematoda), *Ergasilus versicolor* Wilson C.B., 1911 / *E. lizae* Krøyer, 1863 (Copepoda) and *Contracaecum* sp. Larvae III (Nematoda). These parasites had an average prevalence of 48%, 100%, 16%, and mean abundance of 4.56, 8.04, and 0.32. Only the total length of *M. curema* was associated with the average intensity of infection of *A.* (*Phagicola*) *longa*. The metazoan parasites *Ascocotyle* (*Phagicola*) *longa*, *E. versicolor* and *E. lizae* are reported for the first time for *M. curema* from Peru.

Key words: Ecology, epidemiology, helminths, parasitology, trematodes.

Resumen: Metazoos parásitos de la lisa plateada *Mugil curema* Valenciennes, 1836 (Perciformes, Mugilidae) de los humedales de Pantanos de Villa, Lima, Perú. El objetivo del presente trabajo fue evaluar la comunidad de parásitos metazoarios de la lisa plateada *Mugil curema* Valenciennes, 1836 en los humedales de Pantanos de Villa, Lima, Perú. Durante septiembre a octubre de 2012 se recolectaron veinticinco ejemplares de *M. curema*. Los peces presentaron un peso y una longitud promedio de 947,7 ± 513 g y 30,2 ± 5,84 cm, respectivamente. Los parásitos se identificaron y evaluaron utilizando protocolos parasitológicos estándar. Se recolectaron un total de 323 parásitos metazoos con una abundancia total media de 12,92 por pez. El 100% de las lisas plateadas estuvieron parasitadas. Se observó infección con una, dos, tres especies de parásitos, en diez (40%), catorce (56%) y uno (4%) hospedadores, respectivamente. Los tres parásitos encontrados fueron metacercarias de *Ascocotyle* (*Phagicola*) *longa* Ransom, 1920 (Trematoda), *Ergasilus versicolor* Wilson C.B., 1911 / *E. lizae* Krøyer, 1863 (Copepoda) y *Contracaecum* sp. Larvas III (Nematoda). Estos parásitos tuvieron una prevalencia promedio del 48%; 100%, 16% y abundancia media de 4,56; 8,04 y 0,32, respectivamente. Solo, la longitud total de *M. curema* se asoció solo con la intensidad media de infección de *A.* (*Phagicola*) *longa*. Los metazoos parásitos *Ascocotyle* (*Phagicola*) *longa*, *E. versicolor* y *E. lizae* son nuevos registros para *M. curema* para el Perú.

Palabras clave: Ecología, epidemiología, helmintos, parasitología, trematosis.

Introduction

Community studies form the basis of any parasitological study and are useful for making comparisons between host species by their parasitological descriptors to evaluate biodiversity loss or pollution indicators (Poulin 2011, Lafferty 2012, Madhavi & Lakshmi 2012). Most studies of these biological systems consist of interpreting patterns of distribution and abundance of parasitic taxa as hosts and of variables such as their ontogenetic stage (Ferrer-Castelló *et al.* 2007).

One of the most suitable models to study parasites' ecological aspects is the aquatic system of fish's metazoan parasites (Luque & Poulin 2007). For ease of collection of hosts and the possibility of obtaining many replicates, fish are the most studied hosts compared to any other group of vertebrates (Luque & Poulin 2008, Luque *et al*. 2016). Many variables of fish hosts (intrinsic characteristics) and environmental (extrinsic) influence parasitic communities (Poulin *et al*. 2016). The latter has been used to discriminate host populations, trophic interactions, and identify contaminated environments (Muñoz & Cribb 2006, Pulido & Monks 2008).

Mugil curema Valenciennes, 1836 is a fish that is distributed along the east coast of the Atlantic Ocean from northern Spain to Namibia, the entire west coast of the Atlantic including the Caribbean Sea and the Gulf of Mexico, as well as the eastern coast of the Pacific Ocean since The Gulf of California to Chile (Froese & Pauly 2020). Morphological, ecological, and phylogenetic studies have been carried out with *M. curema* (Cicero *et al*. 2020, Neves *et al*. 2020a, b). Recently, phylogenetic studies have shown that the complex of species of *M. curema* includes, at least, four "*M. curema*", considered as cryptic species, and the need for a morphological analysis is suggested to assign a species name to the Pacific lineage from the United States to Ecuador and Peru (Nirchio *et al*. 2017).

Parasitological studies carried out in *M. curema* have been extensive and well documented in South American countries as well as in the rest of the world, as well as the zoonotic potential of its parasites (Fajer-Ávila *et al*. 2006, Oliveira *et al.* 2007, Moreno *et al*. 2008, Santos *et al*. 2013, Luque *et al*. 2016, Castellanos *et al*. 2020). However, in Peru, there is only one registered parasite, *Contracaecum* sp. in *M. curema* (Luque *et al.* 2016) and no records of other species of metazoan parasites on this host. This study aims to evaluate the community of metazoan parasites of the white mullet *M. curema* in the wetlands of Pantanos de Villa, Lima, Peru.

Material and methods

Collection of material and processing of the samples: Twenty-five specimens of *M. curema* were necropsied between September to October 2012 from the Pantanos de Villa Wildlife Refuge, Lima, Peru (12°12′49″S; 76°59′20″O) to study their community of metazoan parasites (Eiras *et al*. 2006). Nineteen specimens were obtained from Laguna Mayor and six from Laguna Marvilla. Sixteen white mullets were obtained in September and nine in October 2012. Fishes were collected in two points of each lagoon using gillnets with 25 mm meshes. The nets were randomly and transversely placed in each lagoon for ten hours. They were checked every 60 minutes and then relocated to the lab (UNMSM-MHN 2014). The average physical-chemical characteristics of both lagoons of the wetlands of Villa, Lima, Peru evaluated *in situ* were: temperature 23 ± 8.49 ° C, pH 8.65 ± 0.35, Electrical conductivity (EC) 7.30 ± 0.35 mS · cm^{-1}, Surface dissolved oxygen (DO) 4.06 ± 0.36 mg · L^{-1}, Background dissolved oxygen (DO) 2.48 ± 0.81 mgL^{-1}, using a multiparameter HANNA® (HI98130, Solitec, Lima, Peru) and the transparency of the lagoon water was 37.45 ± 18.3 cm.

We measured weight (0.1 g) and length (0.1 cm) of the captured fish. All fish underwent a systematic external and internal examination in tissues including skin, fins, gills, eyes (lens and vitreous humor), body cavity, mesentery, and visceral organs (stomach, intestine, liver, swimbladder, heart, and gonads). Parasites recovered were fixed and preserved using methods commonly applied (Özer *et al*. 2016). Identified parasites were grouped as follows: (1) Trematoda (T), (2) Copepod (C), and (3) Nematoda (N) and used for comparing the effects on fish health status at the higher taxonomic level.

Analysis of the samples: The metazoan parasite community's ecological approximation was made to component and infra-community levels (Esch *et al*. 1990). For the case of parasitic species with prevalence's greater than 10% (Esch *et al*. 1990), the dispersion indices (ID) were used to determine the type of spatial distribution of the parasitic populations, Poulin discrepancy (DP), and K of the negative binomial equation with its respective Chi-square value (X^2) to determine the degree of aggregation (Bego & Von-Zuben 2010). The calculations were performed using the statistical package Quantitative Parasitology 3.0 (Rózsa *et al*. 2000). The parasite species alpha diversity was calculated for component community and infracommunity level using the Margalef diversity index (Dmg), the Brillouin index (H) because each fish analyzed corresponded to a fully censused community (Zar 2014). For dominance, the Berger-Parker index (BP) and Simpson Dominance index (D) (Bautista-Hernández *et al*. 2013) were used. The expected species richness was calculated with the Chao-1 index. Pearson's correlation coefficient rp was used to indicate the relationship between the host's total length and parasite abundance. Spearman's rank correlation coefficient r_s was calculated to determine a possible correlation between the total length of host and parasite prevalence, with previous arcsine transformation of the prevalence data (Bautista-Hernández *et al*. 2013, Zar 2014).

The ecological terminology used follows Bush *et al.* (1997). Statistical significance level was evaluated at $p \leq 0.05$. The parasite specimens collected in this study were deposited in the Helminths Parasites and Related Invertebrates Collection - HPIA, of the zoological collection of the Natural History Museum of the Federico Villarreal National University - MUFV, Lima, Peru; the codes are shown in Table I.

Weight–length relationship (WLR): The relationship between the length and weight measurements of 25 white mullets was modeled using the quadratic equation: $W = a+b_1L+b_2L^2$, where W = weight of fish (in g), L = length of fish (in cm), a is a scaling coefficient, and b is shape parameter (Özer *et al*. 2016).

Relative condition factor (K_n): The theoretically expected weight for a given length was calculated using the estimated weights–length relationship curve. Then, the relative condition factor was obtained by the ratio of observed weight (W) and expected weight (We) as below (Le Cren 1951): $K_n = W/ We$. Mean values of estimated K_n were thus calculated for the fish infected by a particular parasite group alone and also all possible combinations of different parasite groups (co-infection) (Özer *et al*. 2016).

Table I. Ecological descriptors of three parasitic metazoans evaluated in the white mullet *Mugil curema* collected in Pantanos de Villa, Lima, Peru.

Specie parasite	*Ascocotyle (Phagicola) longa*†* Trematoda	*Ergasilus versicolor/ E. lizae* Copepoda	*Contracaecum* sp. Nematoda	Total
Parasitized hosts	12	25	4	25
Prevalence (P) %	48	100	16	100
Mean Abundance (MA) ± SE	4.56 ± 1.03	8.04 ± 0.06	0.32 ± 0.14	12.92 ± 6.08
Mean Intensity (MI) ± SE	9.50 ± 1.49	8.04 ± 0.62	2.00 ± 0.45	12.92 ± 1.22
Stage	Metacercaria	Adult	Larvae III	--
Infection site	Gill	Gill	Mesentery	--
kind of strategy	Core	Core	Secondary	--
Variance/mean ratio (ID)	5.83	1.18	2.53	--
Poulin's discrepancy index (DP)	0.57	0.20	0.86	--
Negative binomial exponent k	NA	47.97	0.16	--
Chi-square test (X^2)	--	0.74	--	--
Length ± SE (cm)	29.78±4,23	30.16±5.84	28.40±6.98	
Weight ± SE (g)	755±269	947±514	616±124	
K_n ± SE	0.94±0,39	1.01±0.43	0.68±0.08	
Voucher MUFV-ZOO	HPIA: 183	HPIA: 115	HPIA: 114	--

na= na means that the maximum likelihood estimate of k could not be computed (the procedure didn't converge). The reason could be (among others) that there is too little data or they aren't aggregated at all. SE = standard error. k_n = relative condition factor. †new geographical record. *new host record.

Log-ratio analysis: Amounts of parasite occurrences on the host should be questioned by their relative usages of the available habitat and host conditions if any effect of co-occurrences is of interest. This brings about analyses of five taxonomic groups of parasites in the form of proportional components, that is, relative abundances, which sum to 1. This type of data is known as 'compositional' or 'closed' data in the literature (Özer *et al*. 2016). Therefore, we first calculated the relative abundances. Zeros were replaced by '1' before the relative abundance calculations. All pairwise log-ratios of relative abundances [e.g., log (Trematoda/Copepoda)] were obtained. In aiding to discover parasites' interrelations with host condition, the correlations between log(K_n) and log-ratios were assessed by Pearson correlation coefficient and statistically tested (Özer *et al*. 2016).

The ethical criteria for biological research in the field were met before, during, and after the research, following Costello *et al.* (2016).

Results

Component community: The average weight and lengths of the fishes were 947 g ± 514 (471-2500) and 30.16 ± 5.84 cm (17.5-41), respectively. Total length (TL) and weight (W) of *M. curema* did not show significant differences between lagoons of the Pantanos de Villa Wildlife Refuge for these two parameters (TL t = 1.98, p = 0.50; W t = 1.01, p = 0.08), so the data was analyzed statistically as a single sample. Three different metazoan parasites were collected: metacercarias of *Ascocotyle* (*Phagicola*) *longa* (Trematoda), a complex congeneric species (*Ergasilus versicolor/E. lizae* (Copepoda) evaluated both species together and *Contracaecum* sp. larvae III (Nematoda) (Table I).

No significant differences were found between species richness (t = 0.68, p = 0.50), mean abundance of infection of metazoan parasites (t = 1.13, p = 0.26), *A.* (*Phagicola*) *longa* (t = 0.03, p = 0.97), *E. versicolor* / *E. lizae* (t = 2.32, p = 0.07) and finally for *Contracaecum* sp. (t = 0.47; p = 0.64) for both lagoons of the Pantanos de Villa Wildlife Refuge.

The complex of species of copepods *E. versicolor/E. lizae* were the most prevalent species (100%), and the most abundant parasite collected (201 individuals, representing 62.23% of all parasites). *A.* (*Phagicola*) *longa* was the species with the highest value of mean intensity (9.50 ± 1.49). In this study, no rare species were found since all species had a prevalence above 10% (Table I). The dispersion index (ID) showed three parasites found in *M. curema* are not homogeneously distributed in this fish population. However, on the contrary, they presented an aggregate or contagious type distribution (ID> 1), which is expected in parasitic species in general. In contrast, the DP index indicates that *Contracaecum* sp. presented a higher degree of aggregation than the other parasitic species, which indicates that this nematode had a higher concentration in few hosts and that most of the hosts were not infected by this species (Table I). The dominance index of Berger-Parker for parasite community was slightly elevated. The average value of Brillouin diversity index (H) was 0.74 (Table II).

Infracommunities: Twenty-five (100%) specimens of *M. curema* were parasitized, with at least one parasite species. Overall, 323 individual parasites were collected, with 14.20 ± 6.14 per host. Infections with one parasite species were found in 10 hosts (40%), biparasitism in 14 hosts (56%), and triparasitism in one host (4%). The total length of *M. curema* was associated only with the MI of *A. longa* infection. For the rest of the parasites, no relationship was found with the total length of *M. curema*. Finally, no significant degree of association was observed between the weight of *M. curema* concerning the P%, AM, and MI of the three metazoan parasites (Table III). Infracommunity Margalef diversity index was low compared to Component community Margalef diversity index. The infracommunity dominance index of Berger-Parker was high compared to Component Community Dominance Berger-Parker index. The average value of Brillouin diversity index (H) was low compared to Component community Brillouin diversity index (Table II).

Table II. Alpha diversity indices of the white mullet *Mugil curema* parasitic community and infracommunities, collected in Pantanos de Villa, Lima, Peru. Dmg: Margalef´; H: Brillouin diversity; D: Dominance Simpson; DB-P: Dominance Berger-Parker.

Diversity indices	Component community	infracommunities
Richness	3	1.64±0,58
Individuals	323	12.92±6.07
Dmg	0.34	0.24±0.21
H	0.74	0.31±0.29
D	0.51	0.74±0.23
DB-P	0.62	0.78±0.21
Chao-1	3	1.64±0.56
Monoparasitism	10 (40%)	
Biparasitism	14 (56%)	
Triparasitism	1 (4%)	

The Chao-1 diversity index (3) indicated that the sampling effort was adequate for the community component, and the richness presented the same value.

The relative condition factor: Weight–length relationship of data with estimates a = 4331.43, b_1 = -287.27 and b_2= 5.60 indicating the overall regression equation of weight–length curve as W= 4331.43-287.27L + 5.60L^2 with R^2= 0.53 and SE (Standard Error) of a = (1268) and b_1 = (86.59) and b_2 = (1.46). This relationship enabled us to calculate the theoretically expected weight (We) and then the relative condition factor as defined before.

Relative condition factor (K_n) and Log-ratio analysis: The relative condition factor was on average less than one for the fish parasitized with *A.* (*Phagicola*) *longa* and *Contracaecum* sp. and close to one for *Ergasilus versicolor* / *E. lizae* (Table I). However, no differences were observed in K_n between parasitized and non-parasitized fish for each of the evaluated parasitic groups and in co-infection (Table IV). A lack of correlation was seen between each parasitic taxa and the relative condition factor (Table IV).

Discussion

The community of metazoan parasites found in *M. curema* in this study was dominated by the copepods *Ergasilus versicolor*/*E. lizae*, both species evaluated together, and a low richness of parasitic species. Similarly, a low richness in *M. curema* was observed by Moutinho & Alves (2014) in Rio de Janeiro, Brazil, where only two species of parasites were reported, *Metamicrocotyla macracantha* (Alexander 1954) and *Floridosentis mugilis* (Machado Filho 1951), being the last, a dominant species. However, in our research, ectoparasite species had a more important presence than endoparasites, the opposite of what was observed by Moutinho & Alves (2014). Other investigations such as that of Fajer-Ávila *et al*. (2006) show a higher species richness in *M. curema* evaluated in the Urias estuary, located near the city of Mazatlan, Sinaloa, Mexico, where seven parasites' morphospecies were reported. Some authors mention that the variability of parasitic species in fish as well as the richness and dominance of these species may be subject and influenced directly or indirectly to intrinsic factors of their definitive hosts such as age, ontogenetic development (Marcogliese 2002, Rybkina *et al*. 2016), type of diet (Marcogliese 2002), the geographical distribution of the hosts (Mentz *et al*. 2016, Prado *et al*. 2017, Minaya *et al*. 2020); and to the influence of extrinsic factors such as intermediate host variability, temporal variability (Poulin 2020), trophic levels (Lafferty *et al*. 2008), to mention a few examples.

The three species of metazoans reported in this study showed a contagious distribution, which, as Iannacone *et al*. (2012) suggest is influenced by factors such as (1) the influence of the evolutionary history of parasitic fish for food and reproductive competition; (2) spatial heterogeneity of the fish habitat that produces differences in susceptibility; (3) prevention of host population collapse due to the effects of parasitism and (4) improvement in the chance of infecting fish.

The association of morphological parameters of *M. curema* and the parasitological indices did not show any degree of association except in the TL and MI of *A.* (*Phagicola*) *longa* (r = -0.97; p = 0.03). Galván-Borja *et al*. (2010) also observed this negative correlation in *Mugil incilis* Hancock, 1830, and they suggest that the parasites could be affecting the growth and physiology of the fish. On the contrary, Öztürk (2013) reports a positive correlation in *Liza aurata* Risso, 1810, probably due to the limited availability of intermediate hosts in cold seasons when most of the fish were juveniles (smaller).

Table III. Correlation between total length (TL) and weight (W) of *Mugil curema*, *versus* prevalence (P), average abundance (MA), and average intensity (MI) parasite, collected in Pantanos de Villa, Lima, Peru.

	Ascocotyle (Phagicola) longa	*Ergasilus versicolor/ E. lizae*	*Contracaecum* sp.
TL *vs* P%	r= -0.17; p= 0.83	r= 0.00; p= 1.00	r= -0.68; p= 0.32
TL *vs* MA	r= -0.75; p= 0.25	r= -0.79; p= 0.21	r= -0.31; p= 0.69
TL *vs* MI	r= -0.97; p= 0.03	r= -0.79; p= 0.21	r= -0.24; p= 0.76
W *vs* P%	r= -0.89; p= 0.11	r= 0.00; p= 1.00	r= -0.72; p= 0.28
W *vs* MA	r= -0.76; p= 0.24	r= 0.89; p= 0.11	r= -0.72; p= 0.28
W *vs* MI	r= -0.69; p= 0.31	r= 0.89; p= 0.11	r= -0.72; p= 0.28

The copepod species *Ergasilus versicolor* / *E. lizae* were present in all the fish evaluated (P = 100%). The parasite-host association of *M. curema* and *Ergasilus versicolor*/*E. lizae* has been previously recorded in different countries such as Mexico (Johnson & Rogers 1973, Fajer-Ávila *et al*. 2006), Brazil (Cavalcanti *et al*. 2011), Puerto Rico (Garcia & Williams 1985), and in the present study, with different salinity concentrations. This adaptability and tolerance to different environments and salinity concentrations were demonstrated by Conroy & Conroy (1986), who tested the survival of *E. lizae* copepods in different salinity concentrations (S=37°/oo, 80%, to 5% sea water in distilled water) and showed survival in more than 90% of individuals.

The trematode *A.* (*Phagicola*) *longa* was also reported in this study, which is considered a potential parasite of zoonotic diseases in humans, acting as accidental hosts (Fried *et al*. 2004; Hung *et al*. 2013; Ruiz-Cabrera *et al*. 2019), but has birds and mammals as definitive hosts (Barros *et al*. 2002, Violante-González *et al*. 2015). Rantsios (2016) and the CDC (2021) do not list *A.* (*Phagicola*) *longa* as zoonotic, and only name *Heterophyes heterophyes* (Siebold, 1853) and other heterophids, without specifically noting that other species are zoonotic when transmitted to humans through food consumption. *A.* (*Phagicola*) *longa* was found in the gills, part of the fish that is eliminated during cleaning and evisceration for its consumption. The low intensity of infection found in *M. curema* makes its zoonotic potential extremely low and cannot be considered here as a possible public health problem. However, the occurrence of *A.* (*Phagicola*) *longa* and its potential as a zoonotic agent is mentioned widely in the scientific literature when it's found in the musculature of fish in South America in countries such as Argentina (Martorelli *et al*. 2012), Brazil (Citti *et al*. 2014, Santos & Borgues 2020), Chile (Manfredi & Oneto 1997, Muñoz & Olmos 2008), Colombia (Galván-Borja *et al*. 2010), Peru (Jara & Escalante 1982), Uruguay (Carnevia *et al*. 2005) and Venezuela (Moreno *et al*. 2008). The literature reported some cases of human infections presumably due to *A.* (*Phagicola*) *longa* in Brazil by Chai & Jung (2017) and they consider this species zoonotic. In addition, Devleesschauwer *et al*. (2017), consider that members of the Heterophyidae family, which are transmitted through food, represent an extremely low scale of risk to human health.

Mugilidae fish from estuaries and coastal lakes become infected with the cercariae of *A.* (*Phagicola*) *longa* due to the presence of an intermediate host such as the mollusk *Heleobia australis* (d'Orbigny 1835) in which the parasite develops its redia and cercaria stages (Santos & Borgues 2020). The occurrence with this intermediate host has been mentioned and recorded in Argentina, Brazil, and Uruguay. In Peru, specifically, in the Pantanos de Villa, other mollusk species are reported, including *Heleobia cumingii* (d'Orbigny 1835), which could be the intermediate host *A.* (*Phagicola*) *longa* in this ecosystem. The potential of *H. cumingii* as an intermediate host for cercariae has been demonstrated by Larrea *et al*. (1994), in which he indicates up to four morphotypes of cercariae in *H. cumingii.*

Parasitosis of the metacercariae of *A.* (*Phagicola*) *longa* is not only restricted to the gill areas of the fish as it was in this study, but it is also reported in various sections and organs of the body of their hosts in which high infection rates and prevalence of up to 100% of evaluated fish (Oliveira *et al*. 2007, Moreno *et al*. 2008, Santos *et al*. 2013). It is also important to mention that the low prevalence of *A.* (*Phagicola*) *longa*, compared to other studies, in which 100% prevalence is reported, probably is due to the short period (two months) evaluated and sample size. Another reason is the decrease in the population density of the possible intermediate host snail *H. cumingii* through time as has been happening in the ecosystem of the Pantanos de Villa wildlife refuge (Torres-Zevallos *et al*. 2020).

Finally, the nematode *Contracaecum* sp. with low parasitic infection levels in fish from the Pantanos de Villa. This parasite has been previously reported in the same host in coastal lagoons of Mexico, with a prevalence of 100% on each of the evaluated dates (Fajer-Ávila *et al*. 2006) and obtained from local markets Mexico with a prevalence of 41 % (Reyes-Rodríguez *et al*. 2020). The low abundance and prevalence of this parasite are likely due to the contamination or levels of anthropic stress that occurs in Los Pantanos de Villa (Pulido & Bermúdez 2018), which would be generating negative impacts on its intermediate hosts, thus reducing the infectious potential of the nematode (Fajer-Ávila *et al*. 2006).

Negative effects caused by pathogenic parasites on their hosts can be expected; nevertheless, it is difficult to define or quantify whether those effects directly caused any alteration on the well-being of hosts, and this measure has been applied to either individual or, even though in limited numbers, varieties of co-infecting parasites to their fish hosts (Özer *et al*. 2016). The health status based on the relative condition factor of *M. curema* was not affected by the individual presence of *A.* (*Phagicola*) *longa*, *Ergasilus versicolor* / *E. lizae,* and *Contracaecum* sp., or in co-infection.

Regarding whether the sampling effort of 25 individuals of *M. curema* to estimate the parasite diversity is adequate for the period analyzed and the Chao-1 index confirmed it. The sampling effort in this research was similar to Yamada & Takemoto (2017), discussed about the concept of 'magic cutoff' sample size of 30 individuals proposed by Post & Millest (1991). They detect a parasite of 10% prevalence with 95% confidence, and examined many intrinsic and extrinsic factors that could influence the sampling effort necessary, but did not find any patterns that confirm this "magic cutoff". In fact, for some types of fish substantially lower sampling effort, 10 and 20 fish hosts may be sufficient to accurately characterize their parasite community richness. In the Pantanos de Villa, *M. curema* is a migratory, detritivorous species with an absence of rare parasitic species (<10% prevalence) and composed of two central parasite species as *A.* (*Phagicola*) *longa* and *E. versicolor*/*E. lizae*, and as argued by Yamada & Takemoto (2017), this fish species would require less sampling effort to evaluate the parasitic diversity. However, the results in their study may change if conducted over a longer sampling period.

Other authors like Shvydka *et al.* (2018) reported host sample size ranging between 25 and 40 individuals and the abundance median estimates showed minimal bias, but the sampling distribution skewed to the low values. Finally, Marques & Cabral (2007) suggested that an estimation of three parasitological indices (prevalence, mean abundance, and mean intensity), based on samples of different host size, showed that mean values of all indices were similar irrespective of sample size, but could be an underestimation of values for mean abundance and mean intensity were more perceptible in small (<40) sample sizes. Estimates of prevalence were not significantly affected by sample size in the research of Marques & Cabral (2007). Therefore, the next studies should be focused on investigating the optimum sample size for comparative studies in fish parasitology.

Recent molecular phylogenetic analyses have shown that the *M. curema* species complex includes, at least four "*M. curema*" mitochondrial lineages, considered as cryptic species, and is suggested the need for a morphological analysis to assign a species name to the Pacific lineage from the USA to Ecuador and Peru. Therefore, all the parasitic fauna registered in this research could be assigned to this possible new species of *Mugil* (Nirchio *et al*. 2017).

We conclude this work by highlighting the metazoan parasites *A.* (*Phagicola*) *longa*, *E. versicolor,* and *E. lizae* are new records for *M. curema* from Peru. Likewise, we emphasize that ecological studies are important in the use of these parasitic species as indicators of contamination due to their sensitivity to environmental disturbances, which have been evidenced in their common infection patterns compared to other studies carried out in the same host and wetland ecosystems similar to the Pantanos de Villa.

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