

Original paper

Component community of helminth parasites in black cuskeel *Genypterus maculatus* (Tschudi, 1846) from north Peru

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ABSTRACT. In Peru, only 12.7% (n=207) of fish species have had some record as parasitic metazoan hosts. The objective of this investigation was to determine the community of helminth parasites in black cusk-eel *Genypterus maculatus* (Tschudi, 1846) of north Peru. Fifty specimens of *G. maculatus* from Puerto Pimentel, province of Chiclayo, department of Lambayeque, Peru were evaluated. For the analysis of the parasitic community, the parasitological ecological indexes of prevalence (P), mean abundance (MA), mean intensity (MI) of infection, the dispersion indices, the correlation between total length (TL) and sex of fish vs parasitological ecological indexes, were calculated. The component community of the parasitic helminths in the evaluated fish was dominated by the presence of endoparasitic flatworms and absence of ectoparasites. The parasitic fauna of *G. maculatus* included eight endoparasitic species, four trematodes, two cestodes, one acanthocephalan and one nematode species. The species with the highest prevalence and abundance were *Helicometra fasciata* (Rudolphi, 1819), *Scolex pleuronectis* Müller, 1788 and *Corynosoma australe* Johnston, 1937. The prevalence of infection of *C. australe* was found positively related to the total length of *G. maculatus*. The sex of *G. maculatus* was not found associated with the P, MA and MI of any of the helminth parasites. This is the first study of ecological aspect in the parasitic fauna of *G. maculatus* made in Peru.

Keywords: biodiversity, eumetazoan, ichthyoparasitology, marine fish, Ophidiidae

Introduction

The northern zone of the Peruvian sea (between 5°S and 8°S) is one of the three marine zones with the highest species richness recorded in the Humboldt Current system [1]. Parasitic helminths and related invertebrate species are not mentioned, despite being considered as an important component of the planet's biodiversity [2]. New species are being described due to the increase in research in ichthyoparasitology and parasitology of wild fauna in recent decades [3–5]. In Peru there are currently registered 1630 species of fish, including marine and freshwater species [6], of which only 207 species were investigated for parasitic metazoans in records published until May 2016 (12.7%) [7]. That is, approximately 87.3% of the fish species in Peru

have not been studied for parasites and may harbour new records for Peru.

Black cusk-eel *Genypterus maculatus* (Tschudi, 1846) (Ophidiidae), is a demersal, marine, tropical, oviparous species that is distributed in the Pacific from the archipelago of the Cones, Chile to Tumbes, Peru [6]. It is a common species, which inhabits the rock shelves and depths between 65 and 300 m [6]. The diet of *G. maculatus* is composed of crustaceans (stomatopods, decapods, anomurs) and small teleosts (*Coelorhynchus* spp., *Merluccius* spp.) [8].

The first parasitological studies carried out in the black cusk-eel *G. maculatus* are those carried out by George-Nascimento and Carvajal [9] in a study about the new records of anisakid nematodes in Chilean marine fauna. Later, George-Nascimento and Huet [10] record the cestodes: *Anonchocephalus*

chilensis (Riggenbach, 1896) Lühe, 1902; *Hepatoxylon trichiuri* (Holten, 1802); *Nybelinia* sp.; nematodes: *Anisakis* sp., *Cucullanus* sp., *Phocanema* sp.; trematode: *Lecithochirium genypteri* Manter, 1954; cysts of acanthocephala: *Corynosoma* sp., and the copepod *Lepeophtheirus yanezi* Stuardo and Fagetti, 1961, in the black cusk-eel from the cities of Lebu and Dichato, Chile. In Peru, only two species of parasitic cestodes have been recorded in *G. maculatus*: *Adenocephalus pacificus* Nybelin, 1931 (= *Diphyllobothrium pacificum* (Nybelin, 1931) Margolis, 1956) and *Scolex pleuronetis* Müller, 1788, both in their immature forms [7].

Due to the scarce information about parasitological studies in black cusk-eel mentioned above in Peru, the objective of this study was to determine the community of helminth parasites of *G. maculatus* from the North of Peru, the aggregation indices, diversity, infection rates, and the relationship between helminth parasites with prevalence above 10% with the length and sex of the host.

Materials and Methods

Fifty specimens of black cusk-eel *G. maculatus* were acquired in the fishing terminal of the district of Villa María del Triunfo, Lima, Peru, between the years 2017–2018. The fish were captured and brought from Puerto Pimentel, province of Chiclayo, department of Lambayeque, Peru. For the identification of fish species, the taxonomic keys of Chirichigno and Velez [11] were used. Total length (TL) and sex (S) data were taken before fish necropsy. For the collection of parasites, the oral cavity, gills, branchial cavity, body cavity, stomach, small intestine, large intestine, pyloric caeca, mesenteries, gonads, heart, branchial artery, swim bladder, kidneys, liver and spleen of the fish were carefully examined in laboratory. Parasites were collected and preserved in 70% ethyl alcohol [12].

For the taxonomic study, the cestode, trematode and acanthocephalan were stained in carmine acetic acid or in Gomori trichrome, dehydrated in concentrations of 50%, 70%, 90% and 100% ethyl alcohol, cleared in eugenol and mounted in Canada balsam [12]. The nematodes were rinsed in a mixture of ethyl alcohol-phenol for the observation of structures of taxonomic importance. The nomenclature and classification of trematodes was done following to Kohn et al. [13], Bray et al. [14], Blend and Dronen [15], for cestodes Khalil et al. [16], for acanthocephalans Margolis and Kabata

[17], Lisitsyna et al. [18], and finally for nematodes Anderson et al. [19].

For the analysis of the parasitic community, the parasitological ecological indexes of prevalence (P), mean abundance (MA) and mean intensity (MI) of infection were calculated [20,21]. The type of strategy of each parasitic species was evaluated according to prevalence (%), for which species were classified as „core” species for species with prevalence greater than 45%, „secondary” species for prevalence between 10%–45% and „satellite” species for prevalence less than 10% [22]. The index of specific importance (IE) calculated as the importance of each parasitic species in the ecological assembly was used, in order to obtain an integrated index of infection of both ecological descriptors [23]: $IE = P + (MA \times 100)$. Where: IE = index of specific importance, P = prevalence, MA = mean abundance of infection.

In the case of parasitic species with prevalence greater than 10% [24], the dispersion indices, Morisita index, Lloyd's index, Poulin discrepancy and K of the negative binomial equation with its respective Chi square value (χ^2) to determine the type of distribution and degree of aggregation [25]. The calculations were performed using the statistical package Quantitative Parasitology 3.0 [26].

The Spearman correlation coefficient was used to evaluate the correlation between TL and P%, previously transforming the values of P% to square root of arc sine. The correlation coefficient was used to determine the ratio of the TL of the host to the MA and MI of each parasitic species with the entire host sample (i.e. males and females together; n = 50). In all cases, the normalcy of the data was verified using the Kolmogorov-Smirnov test with the Lilliefors modification and the variance homocentricity based on the Levene test. 2x2 contingency tables were used to calculate the degree of association between the sex of the host and P% of each parasite by means of χ^2 and the Likelihood Ratio test. The Student t test was used to compare the MA and MI of each parasite and the sex of the host. The analysis of the parasites in relation to the size and sex of the host was made only for the species with prevalence greater than 10% [24]. The level of significance was evaluated at a level of $\alpha = 0.05$.

The following indexes of alpha diversity were determined: Richness, Simpson, Shannon, Menhinick, Equitability and Chao-1 [23] for the

Table 1. Ecological descriptors of eumetazoan parasites in *Genypterus maculatus* from north Peru

	Parasites	SI	P %	MA ± SE	MI ± SE	IE	TS
Trematoda	<i>Helicometra fasciata</i>	PC	24	2.46 ± 1.24	10.25 ± 2.53	270	secondary
	<i>Pseudopecoelus</i> sp.	SI	4	0.08 ± 0.06	2 ± 0.31	12	satellite
	<i>Pseudopecoelina</i> sp.	SI	4	0.04 ± 0.03	1 ± 0.14	8	satellite
	<i>Aporocotyle kuri</i>	BA	2	0.02 ± 0.02	1 ± 0.14	4	satellite
Cestoda	<i>Nybelinia</i> sp.	M	2	0.02 ± 0.02	1 ± 0.14	4	satellite
	<i>Scolex pleuronectis</i>	M	40	6.2 ± 2.3	15.5 ± 3.64	660	secondary
Nematoda	<i>Anisakis</i> sp.	SI	2	0.02 ± 0.02	1 ± 0.14	4	satellite
Acanthocephala	<i>Corynosoma australe</i>	M	68	11.1 ± 3.93	16.32 ± 4.76	1178	core

Explanations: SI = site of infection; BA = branchial artery, PC = pyloric caeca, SI = small intestine, M = mesentery; P % = prevalence; MA = mean abundance of infection; MI = mean intensity of infection; IE = specific importance; SE = standard error; TS = type of strategy of each parasitic species

parasitic community component, and for males and females hosts.

For the determination of descriptive and inferential statistics, the statistical package IBM SPSS Statistics 24 was used.

All the helminth specimens studied were deposited in the Colección de Helminthos parásitos e invertebrados afines of the Museo de Historia Natural, Universidad Nacional Federico Villarreal, Lima, Peru (MUFV: ZOO: HPIA: 23-30).

Results

The population structure of black cusk-eel *G. maculatus* was composed of 26 males (52%) and 24 females (48%). The TL of this fish was 30–48 cm

(Mean ± SD = 39.83 ± 4.87 cm). The males of the black cusk-eel were 30–48 cm (39.73 ± 5.79 cm) long and the females 31.5–46 cm (39.94 ± 3.74 cm) long.

The parasite community in the black cusk-eel was composed in its entirety by endoparasites, trematodes, cestodes, acanthocephalans and nematodes, no ectoparasitic monogeneans were recorded.

The parasitic assemblage in *G. maculatus* recorded eight species of which five species had prevalence below 10%, that is, more than half of the species recorded were rare or satellites. The total prevalence of *G. maculatus* infected with at least one parasitic species was 84% (n=42). The parasitic species with the highest prevalence and abundance

Table 2. Aggregation indices to evaluate the dispersion of the most prevalent parasites in *Genypterus maculatus* from north Peru

Aggregation indices	<i>Corynosoma australe</i>	<i>Scolex pleuronectis</i>	<i>Helicometra fasciata</i>
Dispersion Index	69.47	42.84	31.26
p/interpretation	244.16/A	137.88/A	71.75/A
Morisita Index	78.32	47.34	32.36
interpretation	A	A	A
Lloyd's Index	79.57	48.04	32.72
interpretation	A	A	A
Poulin discrepancy	0.79	0.84	0.9
interpretation	A	A	A
Negative binomial exponent (K)	0.27	0.13	0.08
p/interpretation	0.31/A	0.27/A	0.32/A

Explanations: Poulin discrepancy: 0 (absence of aggregation) to 1 (aggregation to the limit); p = value of significance of the Chi square test; A = aggregate

Table 3. Inferential statistics of size parameter and sex of *Genypterus maculatus* from north Peru with parasitological indexes

		Parasites		
		<i>Corynosoma australe</i>	<i>Scolex pleuronectis</i>	<i>Helicometra fasciata</i>
TL vs P	r (Spearman)	0.88	0.37	-0.05
	p	0.02	0.47	0.92
TL vs MA	r (Pearson)	0.21	0.07	0.12
	P	0.14	0.64	0.4
TL vs MI	r (Pearson)	0.21	0.04	0.22
	p	0.22	0.87	0.48
Sex vs P	χ^2	0.04	0.12	0.25
	p	0.85	0.73	0.61
Sex vs MA	t student	0.46	1.35	-0.99
	p	0.65	0.19	0.32
	F (Levene)	0.87	5.99	4.76
Sex vs MI	p	0.36	0.02	0.03
	t student	0.42	1.34	-1.49
	p	0.68	0.19	0.17
	F (Levene)	0.92	2.23 2	8.36
	p	0.34	0.15	0.00

Explanations: TL = total length; MA = mean abundance of infection; MI = mean intensity of infection; P = prevalence; F = Levene test; p = level of significance; r (Spearman) = Spearman correlation; r (Pearson) = Pearson correlation; t = Student's t test; χ^2 = Chi square test

were the trematode *Helicometra fasciata* (Rudolphi, 1819) Odhner, 1902; the cestode larvae *S. pleuronectis* and the acanthocephalan *C. australe* Johnston, 1937. Only *C. australe* had prevalence above 45% and was considered a core species (Table 1). In this study, *A. kuri* is considered a rare species (1/50) because of the low values in the ecological-indexes.

Table 2 shows the analysis of aggregation for secondary species and core species. The dispersion index (DI), Morisita index (MI) and the Lloyd index (LI) indicate a distribution of the aggregation type for the 10 species of eumetazoans.

The total length (TL) of *G. maculatus* was observed to be correlated with the prevalence of

infection of *C. australe*. The sex of *G. maculatus* was not found associated with the P, MA and MI of any of the helminth parasites (Table 3).

The values of the alpha diversity indices of the community component of parasitic metazoans according to sex and the total population of *G. maculatus* is shown in table 4. In comparison to the sexes, only in the Menhinick diversity index and the nonparametric Chao-1 estimator show lower values in the females of this fish. The total community component in *G. maculatus* did not show high or low values in dominance according to Simpson, or in the equitability indexes according to Equitability and Shannon.

The estimator of Chao-1 estimates that the expected richness in *G. maculatus* was 10 species; in this study were found 8 species (Table 4).

In addition, a checklist of the parasitic records of black cusk-eel *G. maculatus* carried out in America was prepared up to December 2019.

Table 4. Alpha diversity for eumetazoans parasites according to sex and population in *Genypterus maculatus* from north Peru

Alpha diversity	<i>Genypterus maculatus</i>		
	total	male	female
Richness S	8	7	5
Individuals	997	612	385
Menhinick	0.25	0.28	0.25
Shannon H	1.00	0.92	1.00
Simpson λ	0.42	0.45	0.42
Equitability J	0.48	0.47	0.62
Chao-1	9.5	10	6

Discussion

The community component of the parasitic eumetazoan fauna in the evaluated fish was dominated by the presence of endoparasitic flatworms and absence of ectoparasites. According

Table 5. Summary of the parasitic records in *Genypterus maculatus*, in South America

Parasites	Site of infection	Location	Reference
Trematoda			
<i>Aporocotyle kuri</i>	BA	Chile, Peru†	[49], present study
<i>Helicometrina nimia</i>	SI	Chile	[50]
<i>Lecithochirium genypteri</i>		Chile	[10,51]
<i>Helicometra fasciata</i>	PC	Peru†	present study
<i>Pseudopecoelus</i> sp.	SI	Peru†	present study
<i>Pseudopecoelina</i> sp.	SI	Peru†	present study
Cestoda			
<i>Anonchocephalus chilensis</i>	SI	Chile	[5]
<i>Adenocephalus pacificus</i>	VS	Peru	[7]
<i>Diphyllobothrium</i> sp.	BCa, M	Chile, Peru	[7,51]
<i>Hepatoxylon trichiuri</i>	G, M, Mu	Chile	[5]
<i>Nybelinia</i> sp.	M	Chile, Peru†	[51], present study
" <i>Scolex pleuronectis</i> "	M	Chile, Peru	[5,7], present study
Acanthocephala			
<i>Corynosoma</i> sp.	M	Chile	[51]
<i>Corynosoma australe</i>	M	Peru†	present study
Nematoda			
<i>Anisakis</i> sp.	SI	Chile, Peru†	[51], present study
<i>Phocanema</i> sp.	L, Mu	Chile	[51]
<i>Cucullanus genypteri</i>	SI	Chile	[5]
<i>Pseudoterranova</i> sp.	not reported	Chile	[51]
<i>Pseudoterranova cattani</i>	not reported	Chile	[5]
Crustacea			
<i>Lepeophtheirus yañezi</i>	BC, Sk	Chile	[5]

Explanations: † registered in the present study; BA = branchial artery; BC = branchial cavity; PC = pyloric caeca; BCa = body cavity; G = gonads; L = liver; SI = small intestine; M = mesenteries; Mu = muscles; Sk = skin, VS = visceral surface

to Poulin [27] the endoparasite communities of fish can be determined by the feeding habits of the hosts, e.g., specialized predators versus generalists, and their ontogenetic changes, as well as by the availability of different species of prey (intermediate hosts) in a given environment. In the case of ectoparasites, this transmission is affected by the environmental characteristics (for example, depth and temperature) [27–29]. In this last factor, the marine depth is usually related inversely to the temperature, i.e., deeper lower temperature in depth aquatic ecosystems, so demersal fish such as black cusk-eel inhabiting depths of 60–300 m were not infected with ectoparasites. Marcogliese [30] suggested that parasitic diversity decreases in

greater depths in marine ecosystems due to the variation of horizontal gradients in salinity, temperature, nutrients and light. This hypothesis could explain the absence of ectoparasites.

This study considers *G. maculatus* as intermediary hosts in the life cycle of *C. australe* and of the tetraphyllid metacestodes *S. pleuronectis*. The presence of parasites in the larval stage can be considered as a signal of an intermediate level in the food web of *G. maculatus*, as shown by Bahamonde and Zavala [8], who found that the diet of *G. maculatus* is composed of crustaceans (stomatopods, decapods, anomurs) and small teleosts (*Coelorhynchus* spp., *Merluccius* spp.).

In all species of eumetazoans with prevalence

greater than 10%, the aggregation indexes showed a contagious distribution, which is influenced by intrinsic and extrinsic factors such as: (a) spatial heterogeneity of the fish habitat that produces differences in susceptibility; (b) influence on the evolutionary history of the parasite for food, spatial and reproductive competence; (c) improvement in the opportunity to infect the fish, and (d) prevention of the collapse of the host population due to the effects of parasitism [31].

Regarding the degree of association between the P, MA and MI of the parasites and the body length of *G. maculatus*, they were not related. Poulin [32] indicates that larger hosts can provide a greater supply of nutrients to parasites and, consequently, would be the most susceptible to greater diversity and parasite burden. This argument could explain the positive relationship observed between the TL of *G. maculatus* influenced the P of *C. australe*.

George-Nascimento and Huet [10], in an ecological-parasitic study of *G. maculatus* collected from central-southern Chile, observed a positive relationship between the size of this host and the abundance of *Anisakis* sp. These authors explain that the parasitosis of these species is a process of accumulation in the host, since the parasites being in the coelomic cavity of the host, cannot escape it. In our study MA has not been influenced by the size of the fish, but by sex. The preference to one of the host sexes could be attributed to the difference in the ecological relationships (behaviour, habitat and diet) of male and female fish [33]. The richness of trematodes in *G. maculatus* in this study consisted of four species. In Chile this richness is lower, since only three species of trematodes have been recorded in the last 34 years (Table 5) of which only *Aporocotyle kuri* is the only trematode species in common in the same host species in both countries [10].

A. kuri has only been registered for the Chilean coast, this being the first record for the coast of Peru.

Another trematode observed in *G. maculatus* is *Pseudopecoelus* sp. which had already been reported by González and Poulin [34] in *Sebastes capensis* (Gmelin, 1788) collected in the town of Huacho, Lima, Peru. This is the only record of this digenean species from Peru and the only ecological data are of MA=0.1; P=10%. Like *A. kuri*, the digenean *Pseudopecoelus* sp. and *Pseudopecoelina* sp. they are considered rare species in the parasitic community of *G. maculatus*. However, this is the

first record of these two genera in *G. maculatus*, so in a later study more information could be given about ecological aspects of these species.

The generalist trematode *H. fasciata* has also been recorded in this study, which presents numerous studies in which fluctuating values are observed in the ecological indexes. See for instance Iannacone et al. [35], who point out a negative correlation between parasitic abundance with the size of the *Sciaena deliciosa* (Tschudi, 1846) host and prevalence below 10%. In the research of Iannacone et al. [35] a strong positive dependence of the P of *H. fasciata* is detected with the length of the black cusk-eel. However, Iannacone et al. [36] do not observe any correlation between the morphological characters of the Chalapo clinid *Labrisomus philippii* (Steindachner, 1866) with the ecological indexes of *H. fasciata* but do report prevalence above 10%. This last study is in agreement with ours, since no association of sex and host size was observed with the MI and AM of *H. fasciata*.

H. fasciata has a wide distribution ranging from distant waters of the North and South Pacific Ocean, from cold seas to tropical waters with a wide range of latitudes, including the Mediterranean Sea, the North Atlantic, Southeast Africa, the Mexican Pacific, Japan, the Caribbean, Tasmania and New Caledonia [15,37]. This wide distribution includes Peru where 10 fish hosts have been reported for this digenean [7]. *H. fasciata* is not only a cosmopolitan species but also a species of wide range of hosts that includes 50 families and 144 species of definitive fish hosts [15]. Only *Ophidion barbatum* Lin., 1758 in the Ophidiidae family that is registered as a host of *H. fasciata* [15]. This study includes the second record of this parasite in this family. The first record of *H. fasciata* is from a fish of the genus *Genypterus*, i.e., *G. maculatus*.

Metacestoda larvae of tetraphyllids were found parasitizing *G. maculatus* in high prevalence. Because they are difficult to identify due to the lack of distinguishing features in the morphology of their scolex and other diagnostic characteristics of the adult cestodes, they were grouped under *S. pleuronectis* to encompass the metacestodes that share common characteristics to this cestode order as suggested by Chambers et al. [38]. The numerous ecological articles published in the last 30 years in South America, have focused on host marine teleosts as models and included a high number of unidentified larval records most of which

corresponded to tetraphyllideans named *Scolex* spp. [39]. Among studies conducted in South America, 12 hosts were reported for Peru [7], including *G. maculatus*. The high parasitosis of these larval forms of tetraphyllid cestodes in *G. maculatus* suggests the important role of this fish in the trophic niche as a predator of crustaceans and smaller fish [8], and at the same time as prey of elasmobranch fish and marine mammals.

Another larval form of cestode reported in this study is the trypanorhyncha *Nybelinia* sp. that unlike tetraphyllid larvae, can be accurately identified because their characteristic tentacular armor [40–41]. Like George-Nascimento and Huet [10], we also observed a single individual of *G. maculatus* parasitized by a single specimen of *Nybelinia* sp. This low prevalence was also observed in other marine fish in Peru including *Brama japonica* Hilgendorf, 1878, *Cynoscion analis* (Jenyns, 1842), *Isacia conceptionis* (Cuvier and Valenciennes, 1830), *Merluccius gayi gayi* (Guichenot, 1848), *Paralonchurus peruanus* (Steindachner, 1875), *Sarda chiliensis* (Cuvier, 1832), *S. deliciosa* and *Trachurus murphyi* Nichols, 1920 [42–46].

Like the tetraphyllid larvae, the acanthocephalan *C. australe* were also found in *G. maculatus* in high prevalence. George-Nascimento and Huet [10] in an ecological study of *G. maculatus* observed the parasitosis of the genus *Corynosoma* acanthocephalan; however, these authors only found a single individual in 80 examined hosts considering it a rare species. In our study, however, this species was found in high prevalence and high intensity of infection. The final hosts are usually mammals and seabirds [47–48]. With the results of this study, *G. maculatus* is added as a new host for the specie *C. australe*.

The parasitosis by the nematode *Anisakis* sp. in this study it was very low (1/50). In the studies carried out by George-Nascimento and Carvajal [9] and George-Nascimento and Huet [10], *G. maculatus* collected from the Chilean coasts had a prevalence of infection by *Anisakis* sp. of 20% (4/20) and 25% (20/80), respectively, i.e., they were not considered rare species of parasites of *G. maculatus* from Chile, as in our study. Likewise, the richness of nematode species in the black cusk-eel of the coast of Peru presented only one species unlike the records made since 1980 where up to six species of nematodes have been recorded in Chile [49–51] (Table 5). This difference of the parasitic

load of nematodes in *G. maculatus* collected from Peru and Chile could be attributed to the difference of years between the two studies (give the years here), the geographical distance, the regional temperature difference, seasons of sampling, the increasing temperature resulting from climatic change of the last 20 years, among other factors.

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