

Variations in the fecundity and body size of digenean (Opecoelidae) species parasitizing fishes from Northern Chile

Variaciones en la fecundidad y tamaño corporal de especies digeneas (Opecoelidae) que parasitan peces del norte de Chile

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Resumen.- La historia de vida y estrategias de reproducción de los parásitos muestran una variabilidad significativa asociada con la identidad de hospedador y con factores ambientales. Este estudio evaluó la influencia de las especies hospedadoras sobre algunos rasgos de historia de vida de digeneos, como el tamaño del cuerpo del parásito (PBL) y la fecundidad, de 3 especies: *Helicometra fasciata*, *Helicometrina nimia* y *Neoleburia georgenascimentoi*. Además, se usaron marcadores moleculares (región V4 del gen 18S y CO-I) para confirmar la identidad específica de las especies parásitas en los diferentes hospedadores. Desde enero 2010 hasta junio 2012, 305 peces pertenecientes a *Paralabrax humeralis*, *Acanthistius pictus*, *Labrisomus philippii*, *Prolatilus jugularis* y *Pinguipes chilensis* fueron capturados, por medio de buceo, desde la costa norte de Chile (24°S). En el laboratorio, los peces se midieron y se recolectaron todos sus parásitos. A cada parásito objetivo se le midió la longitud total y el ancho del cuerpo y todos sus huevos extraídos del útero se contabilizaron. Se utilizaron modelos lineales generalizados (GLM) para identificar los factores que afectan la fecundidad de los digeneos. La fecundidad de *H. nimia* fue mayor en *A. pictus* y menor en *L. philippii*; la fecundidad de *H. fasciata* fue mayor en *P. humeralis* y menor en *L. philippii* y la fecundidad de *N. georgenascimentoi* fue mayor en *P. chilensis*. GLM mostraron que la fecundidad es afectada por el PBL y por la especie hospedadora. Las variaciones en PBL y fecundidad están aparentemente asociados con el tamaño de las especies hospedadoras: los parásitos más grandes (y con mayor fecundidad) se registraron en las especies de peces con mayores tamaños corporales (*P. humeralis*; *A. pictus* y *P. chilensis*). No obstante, los análisis moleculares mostraron que *H. nimia* presente en *L. philippii* difiere a las encontradas en *A. pictus* y *P. humeralis*, mientras que *N. georgenascimentoi* presente en *P. jugularis* es distinta a la encontrada en *P. chilensis*; por lo tanto, la identidad específica de los digeneos podría explicar la diferencia en su fecundidad entre especies de hospedadores.

Palabras clave: *Helicometra*, *Helicometrina*, *Neoleburia*, Serranidae, Labrisomidae, Pinguipidae, ADNm

Abstract.- Parasite life history and reproductive strategies show considerable variability associated with host identity and/or environmental factors. In this study, we measured parasite body length (PBL) and fecundity of 3 digenean species: *Helicometra fasciata*, *Helicometrina nimia* and *Neoleburia georgenascimentoi*, in their different host species, to evaluate the host influence on these biological traits. Additionally, parasite identifications were confirmed using molecular markers (V4 region of 18S and CO-I genes). From January 2010 to June 2012, 305 fish belonging to *Paralabrax humeralis*, *Acanthistius pictus*, *Labrisomus philippii*, *Prolatilus jugularis* and *Pinguipes chilensis* were captured, by diving, off the northern Chilean coast (24°S). In the laboratory, fish were measured and all their parasites collected. The total length and body width of each individual parasite were measured and all eggs were extracted from the parasites' uterus and counted. Generalized lineal models were used to identify factors affecting fecundity of digeneans. Fecundity of *H. nimia* was highest in *A. pictus* and lowest in individuals from *L. philippii*; fecundity of *H. fasciata* was highest in *P. humeralis* and lowest in *L. philippii* and fecundity of *N. georgenascimentoi* was highest in *P. chilensis*. GLM showed that variations in fecundity of digeneans are explained by host fish species and by PBL. Variations in PBL and fecundity are apparently associated with host species sizes: longer parasites (with more eggs per parasite) were recorded in larger fish species (*P. humeralis*, *A. pictus* and *P. chilensis*). However, molecular analyses showed that *H. nimia* from *L. philippii* should be considered a distinct species from specimens found in *A. pictus* and *P. humeralis*, while *N. georgenascimentoi* from *P. jugularis* should be considered a distinct species from specimens found in *P. chilensis*, which might explain the observed fecundity differences in those host species.

Key words: *Helicometra*, *Helicometrina*, *Neoleburia*, Serranidae, Labrisomidae, Pinguipidae, mDNA

INTRODUCTION

Parasite life history and reproductive strategies show considerable variability associated with host identity (Krasnov *et al.* 2004, Riquelme *et al.* 2006, González *et al.* 2012) or environmental factors (Pietroock & Marcogliese 2003, Poulin 2007). There is evidence that the host immunity can affect parasite fitness (Tschirren *et al.* 2007) and some studies have highlighted its role in controlling helminth populations (Meeusen & Balic 2000). Given that the host immune system represents a strong selective force on parasite success (Morand & Sorci 1998), parasites lacking sufficient adaptations may exhibit depressed fecundity (Trouve *et al.* 1998).

If generalist parasites (= those infecting several host species) need to develop counter-adaptations against the immune systems of many potential hosts, specialist parasites (= those infecting only one or two host species) can allocate more resources to reproduction or other functions, resulting in a trade-off between the number of host species that can be successfully exploited and parasite success in those hosts (Poulin & Mouillot 2004). Consequently, parasite species with low host specificity may have evolved in less advantageous conditions than those that are more host-specific (Sasal *et al.* 1999), which could then affect the life-history strategies of each one; and in turn, determine the host-specific levels of parasite abundance (Krasnov *et al.* 2006). Thus, host specificity takes into account epidemiological indices, such as the percentage of infected host species or the intensity of infection, which may reveal stronger host-parasite interactions (Sasal *et al.* 1999), and host specificity is recognized as a key factor determining the spread of parasitic diseases (Gemmill *et al.* 2000).

Parasite identifications are based almost exclusively on morphological characters. However, over the last decade the use of molecular markers has been applied widely as a powerful tool in taxonomy, systematics and phylogeny, allowing to assess whether or not morphological variations correspond to different parasite species or it corresponds only to phenotypic expression of the same species due to environmental variations (Hebert *et al.* 2004, Radulovici *et al.* 2010).

Off the northern Chilean coast, fish species such as Brick seabass *Acanthistius pictus*, Peruvian rock seabass, *Paralabrax humeralis* (Serranidae) and Chalapo clinid *Labrisomus philippii* (Labrisomidae) are parasitized by 2 digenean species, *Helicometrina nimia* and *Helicometra fasciata*. The serranid species reach similar body length

and overlap in habitats associated with seaweeds, *e.g.*, *Lessonia nigrescens* and *Macrocystis* spp. (Cisternas & Sielfeld 2008); whereas *L. philippii* reach lower body length and inhabits demersal habitats (Medina *et al.* 2004). Similarly, both *Pinguipes chilensis* and *Prolatilus jugularis* (Pinguipidae) are hosts to the digenean *Neoleburia georgenascimentoi*. The latter 2 host species differ in body length and habitat preference (González & Oliva 2008).

In this study, parasite body length and fecundity were measured for these 3 digenean species (*H. fasciata*, *H. nimia* and *N. georgenascimentoi*) in the different host species, to evaluate the influence of the host species on these biological traits and, additionally, parasite identifications were confirmed using molecular markers (V4 region of 18S and CO-I genes). Significant differences in the biological traits among host species can be expected if extrinsic factors are identified as key determinants of reproductive success for these parasites.

MATERIALS AND METHODS

SAMPLING AND DATA COLLECTION

From January 2010 to June 2012, 305 fish belonging to *Paralabrax humeralis*, *Acanthistius pictus*; *Labrisomus philippii*; *Prolatilus jugularis* and *Pinguipes chilensis* were captured, by diving, from northern Chilean coast (Isla Santa María, ISM, 24°S). In the laboratory, the fish were measured and examined, and all parasites were collected using traditional parasitology techniques following González & Poulin (2005). Parasites were sorted, counted, and preserved in 70% alcohol for subsequent analyses prior to being identified based on morphological features, following Bray (2002) and Jones *et al.* (2005), as *Helicometra fasciata*, *Helicometrina nimia* and *Neoleburia georgenascimentoi*. The number of fish examined by host species, the prevalence, means abundance and means intensity of the digeneans (*sensu* Bush *et al.* 1997) in the different host species are summarised in Table 1.

Given that the number of eggs in the uterus can be a snapshot of one continuous process of egg production and release (Herrerias *et al.* 2007), fish samples were obtained at different times during year to avoid potential monthly reproductive peak of the digeneans. The total length was measured from relaxed specimens of the selected digenean parasites using a compound

Table 1. Mean abundance (A), mean intensity and prevalence (P%) of the digeneans *Helicometra fasciata*, *Helicometrina nimia* and *Neoleburia georgenascimentoi* in the different host species from Northern Chilean coast / Abundancia media (A), intensidad media (I) y prevalencia (P%) de los digeneos *Helicometra fasciata*, *Helicometrina nimia* and *Neoleburia georgenascimentoi* en los diferentes especies hospedadoras del norte de Chile

Host fish species	Digenean species								
	<i>Helicometra fasciata</i>			<i>Helicometrina nimia</i>			<i>Neoleburia georgenascimentoi</i>		
	A	I	P%	A	I	P%	A	I	P%
<i>A. pictus</i> (n= 41)	0.76	6.1	7.3	0.07	1.5	7.3			
<i>P. humeralis</i> (n= 49)	2.33	7.0	40.8	0.35	3.6	10.2			
<i>L. philippii</i> (n= 70)	0.65	3.0	18.2	3.47	6.1	57.5			
<i>P. jugularis</i> (n= 72)							1.17	3.00	29.4
<i>P. chilensis</i> (n= 64)							0.76	4.08	18.8

microscope. Eggs were extracted from the uterus of each of the target parasites and counted. Fecundity was defined as total number of eggs per parasite individual.

STATISTICAL ANALYSES

In total, 550 parasites were measured (212 of *H. fasciata*, 228 of *H. nimia* and 110 of *N. georgenascimentoi*). The intensity of infection of these digenean species varied between 1 and 18 parasites per fish. Therefore, the median value was used in statistical analyses when there was more than one individual parasite by fish (Table 2).

Because the data set for *H. nimia* and *H. fasciata* did not meet the requirement of homogeneity of variances, Kruskal-Wallis tests were used to evaluate PBL differences among fish species. For *N. georgenascimentoi*, the difference in PBL (log- transformed data) between host species was evaluated with a Student t-test (Zar 1999)

Generalized lineal models (GLM) were used to evaluate fecundity (number of eggs per parasite individual) among fish species, including in the models the variables PBL, fish size and intensity of infection. Given that fish size varied significantly among host fish species, a stepwise model was used to evaluate the effect of. The models were performed using distribution normal for response variable (log-transformed), and as normal link function (Quinn & Keough 2002). All analyses were performed in software STATISTICA 7.0.

MOLECULAR ANALYSES

Recent literature has shown the presence of cryptic parasite species in different hosts. Hence, molecular analyses were performed using region V4 region of the 18S ribosomal RNA (V4) and these were supplemented with partial cytochrome oxidase subunit I (COI) sequence to confirm the identifications of *N. georgenascimentoi*, *Helicometra fasciata* and *Helicometrina nimia* from the different host species.

For molecular analyses, the parasites were isolated and transferred to a 1.5-ml microcentrifuge tube (one in each tube). DNA extraction was performed according to Leung *et al.* (2009). The V4 and partial COI gene were amplified by polymerase chain reaction (PCR) using the primers and methodology (including the amplification protocols) described by Hall *et al.* (1999). The PCR products were visualised on a 1.5% agarose gel and sequenced using an automated capillary electrophoresis sequencer (ABI 3730XL, Macrogen Inc.)¹. The similitude tree reconstruction was performed using the software Mega 5 (Tamura *et al.* 2011), the algorithm Neighbor-Joining (NJ) (Nei & Kumar 2000), and the evolution model Kimura 2-parameter (K2P) (Kimura 1980). Nodal support was assessed using 1000 bootstrap resamplings (Efron 1982). *Lobatostoma manteri* (L16911.1) and *Aspidogaster conchicola* (DQ482608.1) were used as outgroups for the V4. *Haliotrema aurigae* (EU009803.1) and *Macrogryodactylus clarii* (GU252718.1) were used as outgroup for COI. These sequences were obtained from Genbank.

¹Macrogen Inc., Seoul, Korea <<http://www.macrogen.com>>

RESULTS

POPULATION PARAMETERS OF THE DIGENEANS

The highest *H. nimia* prevalence was recorded in *L. philippii*, whereas the highest of *H. fasciata* prevalence was in *P. humeralis*. The lowest prevalences of these digeneans were in *A. pictus*. For *N. georgenascimentoi*, the prevalence was higher in *Prolatilus jugularis* than in *Pinguipes chilensis* (Table 1).

PARASITE BODY LENGTH AND FECUNDITY

The number of individuals measured, range of variations in the parasite body length (PBL), body width and number of eggs per parasite for each digenean species within their host fish species is summarised in Table 2.

PBL of *H. nimia* ($H_{2,165}=74.19, P \ll 0.001$), *H. fasciata* ($H_{2,96}=9.4, P=0.009$) and *N. georgenascimentoi* ($t=2.18, df=69, P=0.03$) varied significantly among host fish species. Individuals of *H. nimia* were longest in *A. pictus* and smallest in *L. philippii*, whereas individuals of *H.*

fasciata were longest in *P. humeralis* and smallest in *L. philippii*. Individuals of *N. georgenascimentoi* were longest in *P. chilensis* (Table 2).

Fecundity of *H. nimia* was highest in *Acanthistius pictus* and lowest in individuals from *L. philippii*, whereas fecundity of *H. fasciata* was highest in *P. humeralis* and lowest in *L. philippii* (Table 2). On the other hand, fecundity of *N. georgenascimentoi* was highest in *P. chilensis* (Table 2). GLM showed that variation in fecundity of digeneans is explained by host fish species and by PBL (Table 3). Consequently, in those host species where the parasites showed longest body length, they also recorded the highest fecundities.

MOLECULAR ANALYSES

For the V4, a total of 9 sequences from *N. georgenascimentoi* (5 sequences from *P. chilensis* and 4 from *P. jugularis*), 10 sequences from *H. fasciata* (2 from *L. philippii*, 5 from *P. humeralis* and 3 from *A. pictus*) and 9 sequences from *H. nimia* (4 from *L. philippii*, 3 from *A. pictus* and 2 from *P. humeralis*) were obtained. All sequences were 414 bp in length.

N. georgenascimentoi of both host fish grouped in the same clade with a bootstrap support of 99%, although the *N. georgenascimentoi* collected from *P. jugularis* were located in one subgroup with a bootstrap support of 65%. Only 0.3% of genetic distance (1 bp difference) was observed between the *N. georgenascimentoi* isolates from *P. chilensis* and *P. jugularis*; there were no significant differences within each subgroup, supporting the specific identity of *N. georgenascimentoi* in both host fish species. Similarly, *H. fasciata* of the 3 host species grouped in the same clade with a bootstrap support of 99%. The genetic distance was only 0.3% (1 bp difference) between sequences from *A. pictus* and the other 2 host species; recording identical sequences of *H. fasciata* between *P. humeralis* and *L. philippii*. On the other hand, *H. nimia* isolated from *A. pictus* and *P. humeralis* grouped in the same clade (99% bootstrap), but those sequences from *L. philippii* were located in a separated group, with 99% bootstrap support. The genetic distance was 2.57% (10 bp difference) between sequences of *H. nimia* from *L. philippii* and those from *A. pictus* and *P. humeralis* (Fig. 1).

For COI, a total of 3 sequences of *H. fasciata*, 3 of *H. nimia* and 8 of *N. georgenascimentoi* were obtained. All sequences were of 737 bp in length. The genetic distance between sequences of *H. fasciata* from *P. humeralis* and *L. philippii* was 0.3% (2 bp difference), both grouped in

Table 2. Mean parasite body length (PBL, mm), mean number of eggs by parasite and mean intensity of the digenean species in the different host species. In parenthesis, is given standard deviation. N= Number of examined individual parasites / Longitud promedio del cuerpo del parásito (PBL, mm), número promedio de huevos por parásito e intensidad media de las especies de digeneos en los diferentes hospedadores. En paréntesis se entrega la desviación estándar. N= número de parásitos examinados

	Host fish species		
	<i>A. pictus</i>	<i>P. humeralis</i>	<i>L. philippii</i>
<i>Helicometrina nimia</i>			
PBL (mm)	5.7 (1.58)	3.3 (1.4)	2.11 (0.5)
Parasite body width (mm)	1.4 (0.35)	0.8 (0.3)	0.6 (0.1)
N° eggs/parasite	326.6 (150.1)	104.9 (85.5)	47.1 (28.1)
Intensity	8.6 (5.6)	5.4 (5.1)	10.1 (5.5)
Fish size (cm)	34.3 (3.0)	35.9 (6.9)	26.1 (3.1)
N	40	46	60
<i>Helicometra fasciata</i>			
PBL (mm)	2.5 (0.6)	2.6 (0.7)	2.1 (0.4)
Parasite body width (mm)	0.6 (0.1)	0.7 (0.2)	0.6 (0.1)
N° eggs/parasite	103.2 (80.4)	150.8 (73.4)	60.2 (43.7)
Intensity	13.5 (6.4)	12.6 (7.8)	3.95 (1.7)
Fish size (cm)	35.8 (3.7)	36.2 (6.9)	26.9 (4.0)
N	43	34	19
<i>N. georgenascimentoi</i>			
	<i>P. jugularis</i>	<i>P. chilensis</i>	
PBL (mm)	2.7 (0.6)	3.2 (0.72)	
Parasite body width (mm)	0.8 (0.2)	0.8 (0.4)	
N° eggs/parasite	152.9 (120.5)	300.1 (217.8)	
Intensity	12.9 (5.4)	5.9 (3.8)	
Fish size (cm)	32.8 (4.0)	41.9 (4.3)	
N	35	42	

Table 3. Results of GLM of the factors that influence the fecundity of digeneans. A) *Helicometra fasciata*; B) *Helicometrina nimia*; C) *Neoleburia georgenascimentoi*. The Chi-square tests assess the significance of the deviance explained by each factor / Resultados del GLM que muestran los factores que afectan la fecundidad de los digeneos, A) *Helicometra fasciata*; B) *Helicometrina nimia*; C) *Neoleburia georgenascimentoi*. El test Chi-cuadrado determina la significancia de la deviance explicada por cada factor

Factor	Degree of Freedom	Chi-Square	P
A)			
Log PBL	1	53.448	<0.001
Log Intensity	1	2.649	0.104
Log Fish size	1	0.089	0.769
Host	2	14.998	0.001
B)			
Log PBL	1	207.45	<0.001
Log Intensity	1	0.269	0.603
Log Fish size	1	5.789	0.020
Host	2	9.66	0.007
C)			
Log PBL	1	15.118	<0.01
Log Intensity	1	0.725	0.39
Log fish size	1	3.128	0.07
Host	1	4.353	0.04

the same clade, with 92% bootstrap support. The genetic distance between the sequences of *H. nimia* from *A. pictus* and *L. philippii* was 21.8% (161 bp difference), being located in two separated clades with 86% and 94% bootstrap support, respectively. The genetic distance between the sequences of *N. georgenascimentoi* from *P. chilensis* and *P. jugularis* was 16.7% (123 bp difference), being located in separated clades with 83% bootstrap support (Fig. 2). Consequently, analyses using both genes support that *H. fasciata* is present in all three fish species studied. However, in the case of *H. nimia*, the genetic differences among sequences from the fish species indicate that there is a different genetic group in *L. philippii* (Figs.1 and 2). On the other hand, the analyses for *N. georgenascimentoi*, using COI, show that each host species (*P. chilensis* and *P. jugularis*) harbour a different genetic group (Fig. 2).

DISCUSSION

Some studies have demonstrated that parasite biological traits, such as body length and fecundity, vary among host species (Poulin 1996, 1999, Krasnov *et al.* 2004,

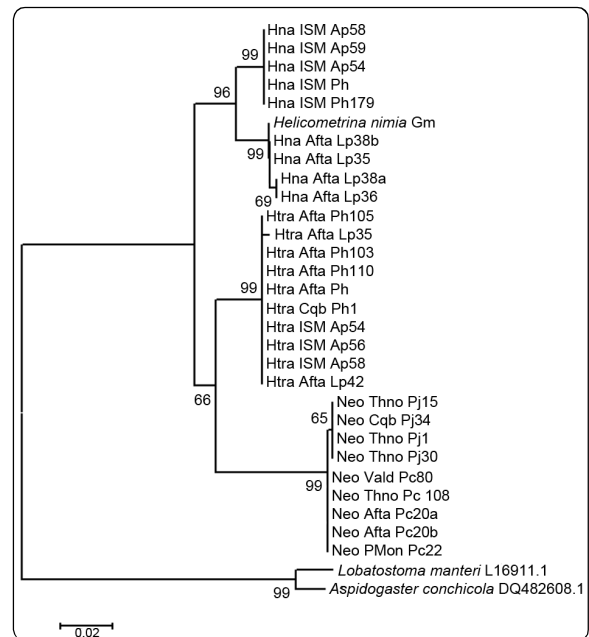


Figure 1. Phylogenetic tree of 3 digenean species present in littoral fish species from Chilean coast, based on V4 region gen 18S using Neighbor Joining algorithm. *Helicometra fasciata* (Htra), *Helicometrina nimia* (Hna) and *Neolebouria georgenascimentoi* (Neo). Fish: *Acanthistius pictus* (Ap), *Gobiesox marmoratus* (Gm), *Labrisomus philippii* (Lp), *Paralabrax humeralis* (Ph), *Pinguipes chilensis* (Pc) and *Prolatilus jugularis* (Pj). Localities: Isla Santa María (ISM, 24°S), Antofagasta (Afta, 24°S), Coquimbo (Cqb, 30°S), Concepción (Conc, 36°S), Talcahuano (Thno, 36°S), Valdivia (Vald, 40°S) and Puerto Montt (PMon, 40°S) / Árbol filogenético de 3 especies digeneas presente en peces litorales de la costa chilena, basado en la región V4 del gen 18S usando el algoritmo Neighbor Joining. *Helicometra fasciata* (Htra), *Helicometrina nimia* (Hna) y *Neolebouria georgenascimentoi* (Neo). Peces: *Acanthistius pictus* (Ap), *Gobiesox marmoratus* (Gm), *Labrisomus philippii* (Lp), *Paralabrax humeralis* (Ph), *Pinguipes chilensis* (Pc) y *Prolatilus jugularis* (Pj). Localidades: Isla Santa María (ISM, 24°S), Antofagasta (Afta, 24°S), Coquimbo (Cqb, 30°S), Concepción (Conc, 36°S), Talcahuano (Thno, 36°S), Valdivia (Vald, 40°S) y Puerto Montt (PMon, 40°S)

Gonzalez *et al.* 2012). However, in one study based on an acanthocephalan/bird relationship, it was found that the fecundity of 2 digenean species of the genus *Diplostomum*, which shared 2 definitive hosts (birds), did not differ between the host species (Karvonen *et al.* 2006). In our study, the digeneans *Helicometra fasciata*, *Helicometrina nimia* and *Neoleburia georgenascimentoi*, recorded high fecundity and large body size, in the largest hosts: *Paralabrax humeralis*, *Acanthistius pictus* and *P. chilensis*, respectively. Whereas *P. jugularis* reach small body sizes, respect to the other fish, and had small parasites with low fecundity. It has been demonstrated

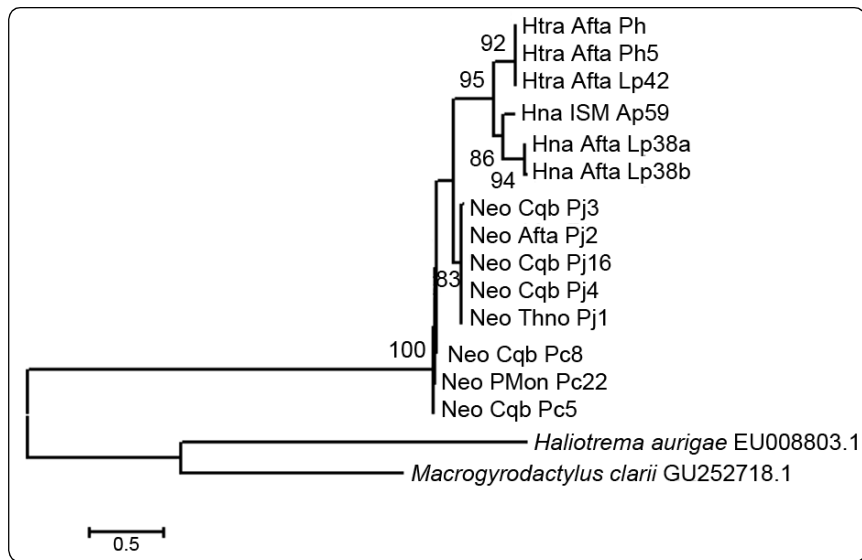


Figure 2. Phylogenetic tree of 3 digenean species present in littoral fish species from Chilean coast, based on gen CO-I using Neighbor Joining algorithm. *Helicometra fasciata* (Htra), *Helicometrina nimia* (Hna) and *Neolebouria georgenascimentoi* (Neo). Fish: *Acanthistius pictus* (Ap), *Labrisomus philippii* (Lp), *Paralabrax humeralis* (Ph), *Pinguipes chilensis* (Pc) and *Prolatilus jugularis* (Pj). Localities: Isla Santa María (ISM, 24°S), Antofagasta (Afta, 24°S), Coquimbo (Cqb, 30°S), Talcahuano (Thno, 36°S), and Puerto Montt (PMon, 40°S) / Árbol filogenético de 3 especies digeneas presente en peces litorales de la costa chilena, basado en el gen CO-I usando el algoritmo Neighbor Joining. *Helicometra fasciata* (Htra), *Helicometrina nimia* (Hna) y *Neolebouria georgenascimentoi* (Neo). Peces: *Acanthistius pictus* (Ap), *Labrisomus philippii* (Lp), *Paralabrax humeralis* (Ph), *Pinguipes chilensis* (Pc) y *Prolatilus jugularis* (Pj). Localidades: Isla Santa María (ISM, 24°S), Antofagasta (Afta, 24°S), Coquimbo (Cqb, 30°S), Talcahuano (Thno, 36°S) y Puerto Montt (PMon, 40°S)

that parasite body size may be constrained by host longevity (Sorci *et al.* 1999) and host size (Morand *et al.* 1996). Thus, the high fecundity and body size of *H. fasciata*, *H. nimia* and *N. georgenascimentoi*, could be mainly explained by host body sizes, however, host habitat, host feeding and/or host behaviour may also have some influence (Morand & Guégan 2000, Poulin 2007, Tschirren *et al.* 2007), for example, *P. chilensis* lives associated with seaweed (*L. trabeculata* and *Macrocystis* spp.), while *P. jugularis* inhabits sandy areas; these fish also have different diets (Medina *et al.* 2004), which may indirectly influences the parasite diets, and consequently affect the parasite fecundity (Heimpel *et al.* 1997).

Inside the host, a parasite is subjected to selective pressures due to food resources (= host nutrients) and host immunocompetence, being suggested that such constraints or advantages could have some influence on the parasite life-history traits (Trouve *et al.* 1998), which, in turn, might determine host-specific levels of parasite abundances (Sassal *et al.* 1999, Krasnov *et al.* 2006). Oliva & Alvarez (2011) studied the relationship between fecundity, worm size, and abundance of the adult digenean *Proctoeces lintoni* (Fellodistomidae) in 4 gastropod hosts (*Fissurella* spp.) and in the clingfish *Sicyases sanguineus*

from northern Chile. These authors recorded that parasites of the keyhole limpet *F. limbata* and the clingfish showed the highest mean fecundity, intensity and prevalence; and therefore, they considered that *P. lintoni* shows the best fitness in those hosts. In our study, *H. fasciata* showed higher fecundity, and higher mean intensity and prevalence, in *P. humeralis*, which could suggest that *H. fasciata* shows the best fitness in *P. humeralis*. This positive relationship between parasite fecundity and mean abundance/prevalence could be explained by increased availability and/or accessibility of infected prey (= intermediate hosts) to fish population of *P. humeralis*. Another plausible explanation can be an increased consumption rate of intermediate prey; thereby, an increased likelihood of getting infected prey (increasing parasite abundance/intensity), and in turn, the host could provides a suitable environment (= more nutrients) that promotes the growth of individual parasites with consequent higher fecundity. Unfortunately, it is not known how many intermediate host species are involved in the life cycle of the digeneans examined, and therefore, we cannot speculate about the role of intermediate host availability in the parasite transmissions.

In the last decades, the use of molecular markers has

been considered a powerful tool in taxonomy, allowing to assess whether or not morphological variations correspond to different species or to phenotypic expressions of the same species due to environmental variability (Hebert *et al.* 2004, Radulovici *et al.* 2010). Our molecular analyses demonstrated that *H. nimia* from *L. philippii* and *N. georgenascimento* from *P. jugularis* and *P. chilensis* are different species; which could explain their significant differences in parasite body sizes and lower mean fecundity in these hosts (see Figs. 1 and 2, Table 2). Nevertheless, *N. georgenascimento* and *H. nimia* showed higher prevalence and mean abundance in *P. jugularis* and *L. philippii*, respectively, which is concordant with host specificity (Sasal *et al.* 1999)

The growth and fecundity of helminths can be affected by density-dependence (Shostak & Scott 1993, Tompkins & Hudson 1999, Dezfuli *et al.* 2002). For example, in an acanthocephalan/fish system, it was observed that egg production by female worms decreased significantly as the infrapopulation size increased, suggesting a density-dependent reduction in female worm fecundity (Hassanine & Al-Jahdali 2008). In our study, generalized lineal models supported that biological traits of the digeneans were not influenced by intensity of infection. On the other hand, GLM showed that in the studied digeneans, fecundity was positively correlated with PBL, which is in concordance with reports for other helminth parasites, such as monogeneans, nematodes and acanthocephalans (Sasal *et al.* 1999, Richards & Lewis 2001, Hassanine & Al-Jahdali 2008).

In summary, body size and consequently fecundity of digeneans varied significantly among host fish species, which is apparently associated with host size species because longer parasites (with more eggs by parasite) were recorded in those longer fish species (*P. humeralis*, *A. pictus* and *P. chilensis*). These results are in agreement with reports for monogeneans (Sasal *et al.* 1999), copepods (González *et al.* 2012) and nematodes (Morand *et al.* 1996, Koprivnikar & Randhawa 2013). However, these results should be cautiously interpreted for the digeneans *H. nimia* (of *L. philippii*) and *N. georgenascimento* (of *P. jugularis*), which correspond to different species as it has been shown with molecular markers. Given the influence that host specificity/generality could have on parasite biological traits; further studies are necessary to advancing in our understanding of the transmission dynamics of the parasites.

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