doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

Veterinaria México OA
Publicación Digital de la Facultad de Medicina Veterinaria y Zootecnia https://veterinariamexico.fmvz.unam.mx/

Identification of ABC genes in monogeneans of the *Ancyrocephalidae* family: an *in silico* and DNA microarray approach

Citlalic Altagracia Pimentel-Acosta[1*]
0000-0002-1854-7843
Daniel Toala-Franco[2]
0000-0002-7797-1169
Víctor Hugo Caña Bozada[3]
0000-0002-8982-8618
Emma Josefina Fájer-Ávila[3]
0000-0003-3230-5709
Francisco Neptalí Morales-Serna[1]
0000-0002-2577-5369

[1] Universidad Nacional Autónoma de México, Instituto de Ciencias del Mar y Limnología/ Laboratorio de Parasitología Acuática, Mazatlán, México.

[2] Universidad Laica "Eloy Alfaro" de Manabí, Facultad de Ciencias del Mar, Manabí, Ecuador.

[3] Centro de Investigación en Alimentación y Desarrollo, A. C. Mazatlán, México.

***Corresponding author**
Email address:
citlalic.pimentel@gmail.com

Abstract

The combination of molecular methods is increasingly efficient for identifying genes in non-model species such as monogeneans. These organisms are parasites that can cause health problems in fish kept in captivity or under farming conditions, making it important to design effective treatments that directly target the parasites' defense systems. ABC (ATP-binding cassette) transporters are proteins involved in the detoxification of xenobiotics and in drug resistance mechanisms. In monogeneans, knowledge related to ABC transporters is limited. In the present study, putative genes encoding ABC proteins were identified in two species of monogeneans, *Scutogyrus longicornis* and *Cichlidogyrus* spp. belonging to the *Ancyrocephalidae* family. For this purpose, transcriptomic data and previously published DNA microarrays were used. These species of monogeneans are commonly found in tilapia farmings. A total of 30 and 59 ABC transporters were predicted in *S. longicornis* and *Cichlidogyrus* spp., respectively. The ABCB and ABCC subfamilies were the most represented. Both species share 19 ABC genes, among which *pgp-1*, *pgp-2*, *pgp-3*, *pgp-9*, *mrp-1*, *mrp-4*, *abce-1*, *abcf-2*, *wht-2*, and *wht-8*, given their relatively higher expression levels, are likely the most important in detoxification processes in *Ancyrocephalidae*. These results could be useful for guiding future experimental work aimed at improving control strategies for monogeneans in fish.

Keywords: Parasites; Platyhelminthes; ABC Transporters; Tilapia; Detoxification.

Submitted: 2023-09-28
Accepted: 2024-04-01
Published: 2024-11-05

Additional information and declarations can be found on page 13

Cite this as:

Pimentel-Acosta CA, Toala-Franco D, Caña Bozada VH, Fájer-Ávila EJ, Morales-Serna FN. Identification of ABC genes in monogeneans of the *Ancyrocephalidae* family: an *in silico* and DNA microarray approach. Veterinaria Mexico OA. 2024;11. doi: 10.22201/fmvz.24486760e.2024.1273.

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

Study contribution

Monogeneans are parasitic helminths that cause health problems in farmed fish, leading to the implementation of anthelmintics to eradicate their presence. However, getting 100 % effective products has not been possible. ABC genes are involved in the detoxification of xenobiotics and could serve as pharmacological targets to develop effective anthelmintics against monogeneans. Nevertheless, information on ABC genes in monogeneans is scarce, probably because they are non-model organisms. In this study, we utilized available molecular information on two species of monogeneans and, by combining *in silico* techniques with DNA microarrays, identified the main ABC genes in two species of monogeneans from the *Ancyrocephalidae* family. Our results contribute to increase the knowledge of ABC genes in monogeneans and can serve as a starting point for studies aimed at improving control strategies for these parasites.

Introduction

Molecular techniques have revolutionized the understanding of physiological processes in organisms. For various species, there is an increasing availability of genomes and transcriptomes, and their analysis helps us better understand complex biological processes. Combining *in silico* and *in vitro* analyses can be useful to carry out *in vivo* experiments, thereby reducing research costs.[1, 2] For instance, in medical research, gene expression analysis of organisms subjected to different conditions has facilitated the identification of genes and metabolic pathways associated with disease or defense mechanisms, as well as potential pharmacological targets.[3–6] This combination of methods can also be useful for predicting biologically important genes in non-model species, where obtaining samples is challenging, such as in the case of monogeneans.

Monogeneans are parasitic helminths, primarily of aquatic organisms, commonly found on the gills of fish. Although most monogenean species are not lethal, some species from the families Gyrodactylidae, Dactylogyridae, and Capsalidae have been responsible for diseases and mortality in marine fish farms.[7] Ancyrocephalidae is another family of monogeneans of interest to the aquaculture sector, as although they are not recognized as lethal, there are species that can cause tissue damage such as the rupture of gill epithelium and blood vessels, leading to cellular infiltration, lamellar hyperplasia, and inflammation, which result in anemia and respiratory problems in freshwater-cultured fish, particularly tilapia.[8, 9]

Tilapia is one of the major freshwater species cultivated worldwide and a source of food and economic income for people in rural communities.[10] Mexico is one of the leading tilapia producers globally, with approximately 169 000 tons produced annually, of which 50 % comes from small-scale farms that contribute to family economies and social stability in vulnerable regions of the country.[11] Tilapia farms in Mexico are not free from parasites, and monogeneans from the family *Ancyrocephalidae*, including *Cichlidogyrus* spp. and *Scutogyrus longicornis*, are quite common.[12–15] The presence of these parasites can cause health issues in tilapia and result in economic losses. In tilapia farms in the southeastern region of the country, it has been observed that the presence of *Cichlidogyrus* spp. and *Scutogyrus* spp. induces anemia in juvenile tilapia with high levels of infection.[16]

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

Several studies have focused on developing anthelmintics to eradicate monogeneans in fish farms; however, methods for determining their efficacy are complicated due to the nonspecificity of the compounds. Occasionally, compounds are found to be effective in eliminating parasites but have negative effects on the fish.[17] Therefore, it is important to continue research to find safe treatments.

For the development of treatments against parasites, it is important to understand the mechanisms related to xenobiotic metabolism, as these are associated with an organism's tolerance to a drug.[18] ABC (ATP-binding cassette) transporters are a group of proteins involved in xenobiotic metabolism. In eukaryotes, these proteins transport xenobiotics from the interior to the exterior of cells across cellular membranes.[18, 19] Generally, ABC transporters have one or two nucleotide-binding domains (NBDs) and one or two transmembrane domains (TMDs). The expulsion process occurs when the NBDs, through ATPase activity, bind and hydrolyze ATP molecules, which produces a conformational change in the protein and allows the transport of the recruited molecule to the exterior of the cell through the TMDs.[20, 21]

In silico identification of genes encoding ABC proteins has been conducted for over 100 helminth species, particularly those of medical and veterinary importance such as nematodes, for which genomic information is available.[21] However, for monogeneans, genomic, transcriptomic, or expressed sequence information is available for only seven species,[22] of which only four have been explored for *in silico* ABC gene identification: *Gyrodactylus salaris* (32 ABC), *Protopolystoma xenopodis* (40 ABC), *Eudiplozoon nipponicum* (46 ABC), and *Neobenedenia melleni* (9 ABC).[23]

Recently, our research group generated the transcriptome of *S. longicornis*,[22] which represents the second available transcriptome for all monogeneans and the first for the *Ancyrocephalidae* family. Based on this transcriptome, the objective of the present study was to identify *in silico* the ABC transporters in *S. longicornis*. Additionally, we verified whether the ABC genes of *S. longicornis* were expressed in heterologous monogenean microarrays of *Cichlidogyrus* obtained from a previous study.[24] This approach is a means to determine the major ABC transporters in the Ancyrocephalidae family, and possibly other monogeneans, involved in the elimination of xenobiotics.

Materials and Methods

Ethics Statement

For this study, approval from the Institutional Ethics Committee was not required as no animals were used.

In silico *identification of ABC genes in Scutogyrus longicornis*

The predicted proteins from the *S. longicornis* transcriptome were downloaded from the Mendeley Data repository.[22] These predicted proteins were aligned against the NBDs of ABC proteins from two model species, *Caenorhabditis elegans* and *Drosophila melanogaster* (InterPro IPR003439), using BLASTp,[25] with an e-value $< 10^{-5}$. Proteins with hits were retrieved and analyzed using MOTIF Search (https://www.genome.jp/tools/motif/MOTIF.html) to determine the topology of each

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

protein (NBD and TMD) according to the domains from the Pfam database.[26] Protein sequences without domains were discarded. Proteins that presented at least one domain were aligned against the non-redundant protein database of the National Center for Biotechnology Information (NCBI) using NCBI BLASTp with default parameters (e-value < 0.05), to classify the proteins by subfamily. According to Sheps et al., metazoans can have eight ABC gene subfamilies (A-H).[27] Additionally, the results of this alignment were used to identify and discard possible contaminant sequences belonging to bacteria or fish. To discard redundant sequences, the longest isoform for each gene was extracted using the Trinity auxiliary script 'get_longest_isoform_seq_per_trinity_gene.pl'.[28]

The subfamily classification of the ABC transporters of *S. longicornis* was confirmed through a phylogenetic analysis. For this purpose, NBD domains were obtained using PS-SCAN v1.86[29] with the Prosite profile PS50893. Domain sequences were aligned using MUSCLE v3.8.31[30] with default parameters. Gaps were trimmed with trimAL v1.4[31] using the automatic mode (-automated1). ModelFinder[32] was used to select the best evolutionary models, and IQ-TREE 1.6.12[33] was used to construct the phylogenetic tree using the approximate likelihood ratio test, which is similar to the Shimodaira-Hasegawa test (1 000 replicates). The tree was visualized using the program FigTree 1.4.2.[34]

Identification of ABC genes in microarrays of Cichlidogyrus *spp.*

The obtention of heterologous microarrays of *Cichlidogyrus* spp. is described in the work of Pimentel-Acosta et al., in which the toxicity of silver nanoparticles (AgNPs) was evaluated.[24] In that study, *Cichlidogyrus* parasites were obtained from the gills of tilapia raised on farms in Sinaloa. These parasites were exposed in vitro to a low concentration (6 µg/L) and a high concentration (36 µg/L) of AgNPs. After one hour of exposure, the monogeneans were collected, fixed, and preserved in RNAlater, followed by RNA extraction using the RNeasy® Plus Micro Kit (QIAGEN). Heterologous microarrays were performed using microarrays with the 20 000 genes of a model species, the nematode *C. elegans*. Gene expression levels under each concentration of AgNPs were statistically calculated using the normalization and Z-score transformation method.[35] The expression profiles of the *Cichlidogyrus* spp. microarrays at 6 µg/L and 36 µg/L of AgNPs are available at http://www.mdpi.com/1422-0067/21/16/5889/s1.

To identify the expression of ABC genes in *Cichlidogyrus* spp., the codes for the 60 ABC genes reported for *C. elegans*[27] were searched in the microarrays, along with the expression levels (z-score) at the two AgNP concentrations (6 µg/L and 36 µg/L).

Homology and differential expression of ABC genes between S. longicornis *and* Cichlidogyrus *spp.*

Using BLASTp, the amino acid sequences of ABC transporters obtained from the transcriptome of *S. longicornis* were aligned against the amino acid sequences of heterologous genes from *Cichlidogyrus* spp., which are genes originally from *C. elegans*. The heterologous sequences were downloaded from NCBI. The best alignment results with an e-value < 10^{-5} were considered homologous proteins

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

between *S. longicornis* and *Cichlidogyrus* spp. To determine the genes that are most actively involved in detoxification processes in the monogeneans, ABC transporters that were overexpressed and underexpressed with $z - score \geq +1$ $y \geq -1$ were considered.

Results

Domain analysis, homology search, and phylogenetic analysis allowed for the identification and classification of 30 ABC transporters in the transcriptome of *S. longicornis*: 1 ABCA, 12 ABCB, 10 ABCC, 1 ABCE, 4 ABCF, and 2 ABCG (Figure 1). No members of the ABCD and ABCH subfamilies were found. The topology of each protein according to its domains is presented in Table 1.

The expression profiles from the microarrays contain z-score values for 19 539 genes at 6 µg/L and 19 472 genes at 36 µg/L of AgNPs, of which 59 correspond to ABC genes in *Cichlidogyrus* spp. (Table 2), grouped into the following subfamilies: 7 ABCA genes, 23 ABCB genes, 9 ABCC genes, 5 ABCD genes, 1 ABCE gene, 3 ABCF genes, 9 ABCG genes, and 2 ABCH genes. Only the gene hmt-1 (W09D6.6) from *C. elegans* was not expressed in the monogeneans, and the gene pgp-3 (ZK455.7) was expressed only in parasites exposed to the higher concentration of AgNPs (36 µg/L). Of the 59 ABC genes, only 22 showed significant differential expression with a z-score of 1 or greater (Figure 2).

Of the 30 predicted transporters in the transcriptome of *S. longicornis*, 19 were homologous to ABC genes expressed in the heterologous microarrays of *Cichlidogyrus* spp. (Table 3), of which 10 showed significant differential expression with a z-score of 1 or greater (Figure 2).

Discussion

In this study, putative ABC transporters were identified in monogeneans representing the family Ancyrocephalidae, with 30 ABC transporters in *S. longicornis* and 59 in *Cichlidogyrus* spp. The number of ABC transporters in *S. longicornis* is similar to the 32 ABC transporters reported in the genome of *G. salaris*, possibly because they belong to the same subclass, Monopisthocotylea. In contrast, other species of monogeneans in the subclass Polyopisthocotylea have a higher number of ABC transporters, such as *P. xenopodis* (40 ABC) and *E. nipponicum* (46 ABC).[23]

However, although *Cichlidogyrus* spp. also belongs to the *Monopisthocotylea* subfamily, we obtained a higher number of ABC transporters, likely due to the genomic technique used, gene hybridization with microarrays, which is very sensitive and can detect very low expression levels (z-score 0.01). However, these genes may sometimes be considered false positives.[36] According to Wurmbach et al., as expression levels decrease, the rate of false positives may increase.[36] It is also possible that the samples from *S. longicornis* and *Cichlidogyrus* spp. were contaminated with DNA from their hosts, which could have generated false positives.

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

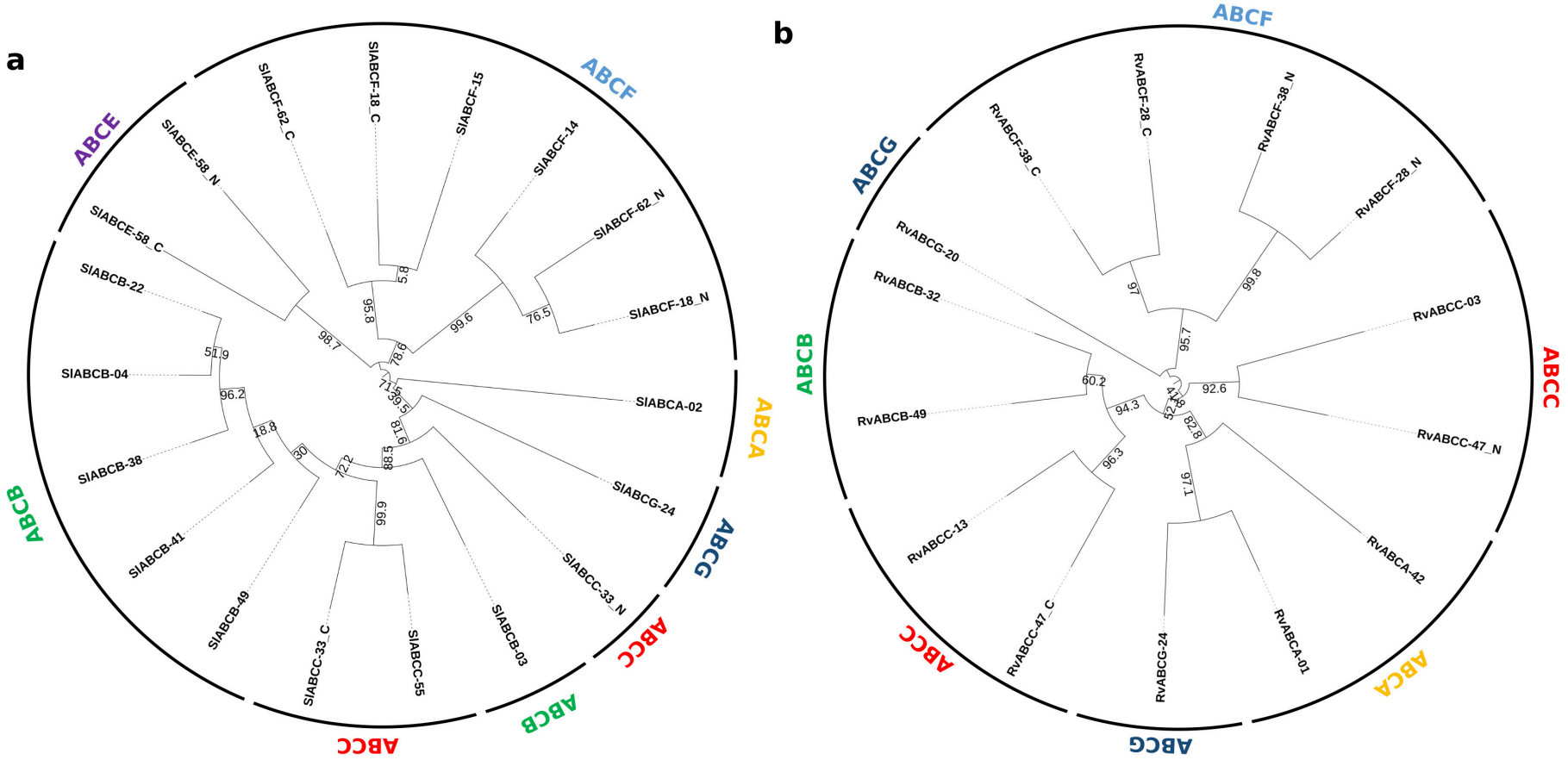

Figure 1. Unrooted phylogenetic tree of NBD domains of ABC proteins from *Scutogyrus longicornis*. The different subfamilies of ABC proteins are indicated in the figure. The LG + I + G4 model was selected. The numbers at the basal nodes represent the frequencies with which the presented tree topology was obtained from 1 000 bootstrap iterations. The scale bar represents one amino acid substitution per site.

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

Table 1. ABC genes identified *in silico* in the transcriptome of *Scutogyrus longicornis*. ID. identifier code; Domain. topology of transmembrane domains (TMD) and nucleotide-binding domains (NBD) found in ABC transporters

Sub-family	ID *Scutogyrus longicornis*	ID tree	Domains	ID of the best alignment to NCBI sequences	e-value
A	DN1173_c0_g1_i1__g.29564	SlABCA-02	TMD-NBD	KAA3669979.1	4.00E-177
B	DN13787_c0_g1_i1__g.67950	SlABCB-04	TMD	KAA0195735.1	1.00E-68
B	DN20467_c0_g1_i1__g.61970	SlABCB-17	NBD	TPP58188.1	6.00E-21
B	DN25_c0_g1_i2__g.15918	SlABCB-22	TMD-NBD	THD20367.1	2.00E-170
B	DN4541_c0_g2_i2__g.852	SlABCB-38	TMD-NBD	KAA3680384.1	2.00E-148
B	DN4924_c0_g2_i3__g.189	SlABCB-45	NBD	CDS30162.2	1.00E-29
B	DN1018_c0_g1_i5__g.51207	SlABCB-01	TMD	AIJ28498.1	1.00E-51
B	DN1516_c1_g4_i1__g.44166	SlABCB-10	NBD-TMD	THD21346.1	1.00E-37
B	DN4541_c0_g3_i1__g.869	SlABCB-41	NBD	CDS15383.1	2.00E-118
B	DN1215_c2_g1_i1__g.35156	SlABCB-03	TMD-NBD	XP_031341751.1	3.00E-123
B	DN1566_c0_g2_i3__g.45160	SlABCB-13	TMD	KAF5397403.1	8.00E-103
B	DN5305_c0_g2_i4__g.14196	SlABCB-49	NBD-TMD	CDS39599.2	9.00E-171
B	DN13787_c0_g2_i1__g.67955	SlABCB-06	NBD-TMD	KAG5449322.1	4.00E-60
C	DN14500_c0_g1_i1__g.26566	SlABCC-09	TMD	GAA49862.1	1.00E-21
C	DN2689_c0_g1_i4__g.61338	SlABCC-26	TMD	THD26585.1	1.00E-17
C	DN3269_c0_g2_i4__g.66293	SlABCC-28	TMD-NBD	THD25957.1	9.00E-125
C	DN3269_c0_g4_i1__g.66285	SlABCC-30	TMD	THD25957.1	4.00E-37
C	DN3711_c0_g2_i1__g.49339	SlABCC-32	TMD-NBD	GAA49862.1	3.00E-29
C	DN9321_c0_g1_i1__g.19656	SlABCC-61	NBD	THD25957.1	2.00E-41
C	DN181_c2_g1_i2__g.48169	SlABCC-16	NBD	CDS40693.1	5.00E-18
C	DN3959_c1_g2_i1__g.57276	SlABCC-35	TMD	XP_018650283.1	1.00E-57
C	DN3852_c0_g1_i2__g.65204	SlABCC-33	TMD-NBD	RTG86932.1	1.00E-162
C	DN7784_c0_g1_i1__g.23933	SlABCC-55	NBD	KAA0187611.1	3.00E-91
E	DN786_c1_g1_i3__g.58120	SlABCE-58	NBD	TNN16384.1	0
F	DN252_c0_g1_i1__g.22598	SlABCF-18	NBD-NBD	KAF5405384.1	0
F	DN1595_c0_g1_i4__g.44956	SlABCF-14	NBD	TNN09356.1	3.00E-156
F	DN1595_c0_g2_i1__g.44958	SlABCF-15	NBD	CAX69408.1	1.00E-114
F	DN957_c0_g1_i11__g.3992	SlABCF-62	NBD-NBD	THD27250.1	0
G	DN2637_c0_g1_i3__g.61615	SlABCG-24	TMD-NBD-TMD	KAG5446220.1	0
G	DN643_c0_g1_i18__g.70696	SlABCG-52	TMD-NBD	KAG5453675.1	7.00E-72

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

Table 2. ABC genes expressed in the heterologous microarrays of *Cichlidogyrus* spp.

Sub-family	ID Cspp	Putative gene symbols	AgNPs 6 µg/L z-score	AgNPs 36 µg/L z-score	Sub-family	ID Cspp	Putative gene symbols	AgNPs 6 µg/L z-score	AgNPs 36 µg/L z-score
A	C24F3.5	abt-1	-0.34	-0.12	B	ZK484.2	haf-9	0.51	-0.45
	C48B4.4	ced-7	-0.81	0.68	C	C18C4.2	cft-1	1.68	1.67
	F12B6.1	abt-2	-0.3	0.18		E03G2.2	mrp-3	0.23	-0.18
	F55G11.9	abt-3	0.04	-1.06		F14F4.3	mrp-5	0.99	0.81
	F56F4.6	F56F4.6	-0.32	0.34		F20B6.3	mrp-6	0.2	0.98
	Y39D8C.1	abt-4	-1.41	-0.95		F21G4.2	mrp-4	4.27	-0.93
	Y53C10A.9	abt-5	-0.43	-0.53		F57C12.4	mrp-2	0.77	0.56
B	C05A9.1	pgp-5	-0.45	0.9		F57C12.5	mrp-1	-1.41	1.02
	C34G6.4	pgp-2	0.61	1.8		Y43F8C.12	mrp-7	0.77	0.3
	C47A10.1	pgp-9	1.28	0.87		Y75B8A.26	mrp-8	0.07	-0.94
	C54D1.1	pgp-10	-0.17	0.61	D	C44B7.8	pmp-1	0.33	0.24
	DH11.3	pgp-11	0.66	0.73		C44B7.9	pmp-2	-0.21	-1.38
	F22E10.1	pgp-12	-0.55	-0.57		C54G10.3	pmp-3	-0.11	-0.46
	F22E10.2	pgp-13	-0.55	0.77		T02D1.5	pmp-4	0.02	-1.17
	F22E10.3	pgp-14	0.88	0.89		T10H9.5	pmp-5	-0.14	-0.4
	F22E10.4	pgp-15	0.49	0.39	E	Y39E4B.1	abce-1	1.22	-0.9
	F42E11.1	pgp-4	-0.98	0.55	F	F18E2.2	abcf-1	0.76	0.37
	K08E7.9	pgp-1	-1.49	0.43		F42A10.1	abcf-3	-0.08	-0.14
	T21E8.1	pgp-6	0.12	-0.55		T27E9.7	bcf-2	0.87	1.89
	T21E8.2	pgp-7	0.01	0.52	G	C05D10.3	wht-1	-0.35	-0.06
	T21E8.3	pgp-8	0.66	-0.34		C10C6.5	wht-2	-1.02	-0.6
	ZK455.7	pgp-3	*	-1.27		C16C10.12	wht-3	-0.22	1.83
	C30H6.6	haf-1	0.5	0.32		F02E11.1	wht-4	0.43	0.33
	F43E2.4	haf-2	0.92	0.18		F19B6.4	wht-5	0.36	0.64
	F57A10.3	haf-3	0.79	-0.28		T26A5.1	wht-6	1.68	-1.17
	W04C9.1	haf-4	-1.35	-1.46		Y42G9A.6	wht-7	0.63	0.54
	W09D6.6	haf-5	*	*		Y47D3A.11	wht-8	0.26	1.22
	Y48G8AL.1	haf-6	1.71	-0.19		Y49E10.9	Y49E10.9	0.36	-0.05
	Y50E8A.16	haf-7	1.18	1.43	H	C56E6.1	abcx-1	-0.07	-0.77
	Y57G11C.1	haf-8	2.59	1.57		C56E6.5	abch-1	-1.23	-1.6

The microarrays were exposed to AgNPs for one hour (+ overexpressed, - underexpressed, * no expression). ID Cspp refers to the identifiers of the heterologous gene sequences from *Cichlidogyrus* spp., which are genes originally from *C. elegans*.

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 2024

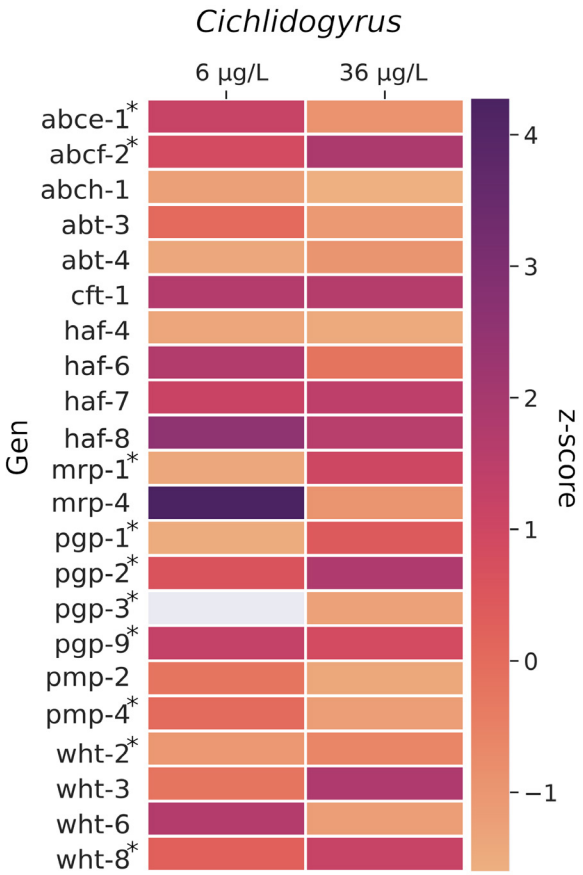

Figure 2. ABC genes with significant differential expression identified in the microarrays of *Cichlidogyrus* spp. ABC genes with homologs in *S. longicornis* are marked with an asterisk (*).

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

Table 3. Shared ABC genes between *Scutogyrus longicornis* and *Cichlidogyrus* spp.

ID Sl	ID Cspp	Putative gene symbols	% Identity	e-value
SlABCB-22	K08E7.9	pgp-1	36.833	4.69E-110
SlABCB-17	K08E7.9	pgp-1	34.545	3.76E-14
SlABCB-38	C34G6.4	pgp-2	36.056	7.62E-94
SlABCB-10	C34G6.4	pgp-2	39.181	2.61E-31
SlABCB-06	C34G6.4	pgp-2	37.582	1.12E-49
SlABCB-04	ZK455.7	pgp-3	56.79	4.79E-53
SlABCB-01	C47A10.1	pgp-9	29.771	7.74E-36
SlABCB-41	F22E10.2	pgp-13	47.561	2.87E-96
SlABCC-33	F57C12.5	mrp-1	34.742	2.36E-109
SlABCC-32	F57C12.5	mrp-1	27.5	4.87E-14
SlABCC-26	F57C12.5	mrp-1	24.893	4.98E-09
SlABCC-55	F21G4.2	mrp-4	59.227	3.62E-87
SlABCC-61	F21G4.2	mrp-4	44.8	6.78E-33
SlABCC-16	F14F4.3	mrp-5	35.632	8.73E-09
SlABCC-09	F20B6.3	mrp-6	36.735	6.71E-13
SlABCC-30	Y43F8C.12	mrp-7	48.739	1.60E-30
SlABCC-37	Y43F8C.12	mrp-7	26.316	5.62E-13
SlABCC-35	Y43F8C.12	mrp-7	31.081	9.45E-29
SlABCC-28	Y75B8A.26	mrp-8	26.027	9.16E-42
SlABCF-18	F18E2.2	abcf-1	54.947	0
SlABCF-14	T27E9.7	abcf-2	53.067	9.12E-117
SlABCF-15	T27E9.7	abcf-2	59.912	4.34E-106
SlABCF-62	F42A10.1	abcf-3	42.779	0
SlABCE-58	Y39E4B.1	abce-1	66.113	0
SlABCA-02	F12B6.1	abt-2	28.016	2.70E-94
SlABCG-24	Y47D3A.11	wht-8	30.192	1.72E-80
SlABCB-03	F57A10.3	haf-3	31.281	6.61E-77
SlABCG-52	C10C6.5	wht-2	33.854	1.39E-26

The percentage of identity and e-value are derived from the alignment of amino acid sequences. ID Sl refers to the identifiers of sequences from *S. longicornis*; ID Cspp refers to the identifiers of the heterologous gene sequences from *Cichlidogyrus* spp., which are genes originally from *C. elegans*.

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

Although sequence filtration was possible in the transcriptome of *S. longicornis* using bioinformatics tools,[22] the microarray results do not allow for this procedure. Another possible explanation is that the exposure to AgNPs in *Cichlidogyrus* spp. may have promoted the activation of more genes compared to *S. longicornis*, whose specimens were not exposed to any xenobiotic. For this reason, the homologous ABC transporters between *S. longicornis* and *Cichlidogyrus* spp. can be considered more reliable for these species of monogeneans from the family *Ancyrocephalidae*.

Similar to this study, other research has also found that the ABCB and ABCC subfamilies are the most represented in helminths.[21, 37] This is likely due to the ability of ABCB transporters or P-glycoproteins (Pgp) and ABCC transporters or MRP genes to expel a wide variety of substrates from cells,[38] including xenobiotics.[39] Although Pgp and MRP genes have a high similarity in amino acid sequence conformation, differing by only 15 %.[40] It has been seen that these transporters are localized in different sites. For example, in the human blood-brain barrier, Pgp genes are expressed in endothelial cells and perivascular astrocytes, whereas MRP genes are found in the epithelial cells of the choroid plexus. Thus, ABC transporters complement each other by acting at different sites,[41] which is why it is common to see both ABCB and ABCC transporters expressed simultaneously in expression profiles.

Identification of 19 putative homologous ABC transporters between *S. longicornis* and *Cichlidogyrus* spp. suggests that these transporters may play a significant role in the detoxification processes of Ancyrocephalidae, particularly the 10 ABC genes: *pgp-1* (-1.49), *pgp-2* (1.80), *pgp-3* (-1.27), *pgp-9* (1.28), *mrp-1* (-1.41), *mrp-4* (4.27), *abce-1* (1.22), *abcf-2* (1.89), *wht-2* (-1.02), and *wht-8* (1.22), which showed relatively higher expression levels with a z-score of 1 or greater. Pgp genes are an important group of ABC genes involved in detoxification mechanisms and are associated with parasite resistance to xenobiotics.[21, 42]

Here, we confirm that in monogeneans, P-glycoproteins (Pgps) are also among the most representative genes, similar to other helminths. In the parasitic nematode *Parascaris univalens*, overexpression of *pgp-2* and *pgp-9* has been documented in individuals exposed to ivermectin, pyrantel, and thiabendazole.[43, 44] Similarly, in the parasitic nematode *Haemonchus contortus*, Williamson et al. observed overexpression of *pgp-2* and *pgp-9* in studies on multidrug resistance.[45] On the other hand, the downregulation of *pgp-1* (-1.49) at 6 µg/L AgNPs and *pgp-3* (-1.27) at 36 µg/L AgNPs may indicate reduced detoxification processes. Kaur and Dey observed decreased resistance to verapamil in Leishmania donovani through the downregulation of pgp-1.[46]

Similarly, Wartenberg et al. mention that the downregulation of Pgp genes is associated with increased levels of reactive oxygen species (ROS) in cells, leading to oxidative stress and consequent damage to organisms.[47] This is consistent with the results of our previous study, where AgNPs activated molecular mechanisms related to ROS generation.[24] It is likely that in the present study, the downregulation of Pgp genes is related to oxidative stress caused by AgNPs in monogenean parasites.

Another important group of ABC transporters in this study were the MRP genes. Previously, it was observed that AgNPs induce the activation of detoxification mechanisms through the overexpression of the *mrp-4* gene in monogeneans exposed to AgNPs (6 µg/L).[24] In the present study, we confirm that *mrp-4* (4.27) was the most significantly overexpressed ABC gene, with a marked difference in expression levels. *Mrp-4* is a member of the ABCC/MRP subfamily of ABC transporters,

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 2024

and its main function is to export endogenous and xenobiotic compounds out of the cell for cellular protection. Studies in various organisms have demonstrated increased expression of MRPs in response to drug exposure.[48]

In *C. elegans*, *mrp-4* has been observed to be involved in lipid transport and storage, but it is also noted for increased expression in ivermectin-resistant organisms.[49] In addition to *mrp-4*, genes *mrp-1*, *mrp-5*, *mrp-6*, *mrp-7*, and *mrp-8* were identified in *S. longicornis* and *Cichlidogyrus* spp., as well as in other helminths such as the nematodes *C. elegans*, *Cooperia oncophora*, and *H. contortus*, where *mrp-1* was overexpressed when exposed to ivermectin and monopantel.[49–51] James et al. suggest that the increase in *mrp-1* expression is associated with the development of resistance in the liver fluke Fasciola spp.[52]

Conclusions

This study expands the current knowledge on the characterization of ABC genes in parasites of the family Ancyrocephalidae, particularly in the species *S. longicornis* and *Cichlidogyrus* spp. Additionally, the work demonstrated the robustness of the employed methodology (a combination of molecular techniques and bioinformatic analysis), which allows for a more comprehensive characterization of ABC genes in the species under study compared to using a single technique.

These results may serve as a starting point for predicting key ABC genes involved in xenobiotic detoxification in other monogenean species. We suggest that the genes *pgp-1*, *pgp-2*, *pgp-3*, *pgp-9*, *mrp-1*, and *mrp-4* may be important for future studies aimed at improving control strategies for these parasites.

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

Data availability

All relevant data are referenced in the document. The predicted proteins from the transcriptome of *Scutogyrus longicornis* are available in the Mendeley data repository, doi: 10.17632/2wnwn4d7p.1. The expression profiles of the microarrays for *Cichlidogyrus* spp. are available at http://www.mdpi.com/1422-0067/21/16/5889/s1.

Funding statement

This research was funded by the National Council of Science and Technology, now the National Council of Humanities, Science, and Technologies (CONAHCYT), through project FORDECYT 292474, awarded to EJ Fájer-Ávila. The funder had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation. During this research, VH Caña-Bozada and CA Pimentel-Acosta received support from CONAHCYT through Doctoral and Postdoctoral scholarships, respectively.

Conflicts of interest

The authors declare that they have no conflict of interest regarding this publication.

Author contributions

Conceptualization: FN Morales-Serna, CA Pimentel-Acosta.
Data Curation: CA Pimentel-Acosta, VH Caña-Bozada, D Toala-Franco.
Formal Analysis: CA Pimentel-Acosta, VH Caña-Bozada, D Toala-Franco.
Funding Acquisition: EJ Fájer-Ávila, FN Morales-Serna.
Investigation: FN Morales-Serna, CA Pimentel-Acosta, VH Caña-Bozada.
Methodology: CA Pimentel-Acosta, VH Caña-Bozada.
Project Administration: EJ Fájer-Ávila, FN Morales-Serna.
Resources: EJ Fájer-Ávila, FN Morales-Serna.
Software: CA Pimentel-Acosta, VH Caña-Bozada, D Toala-Franco.
Supervision: FN Morales-Serna.
Visualization: CA Pimentel-Acosta, D Toala-Franco.
Original Draft Writing: CA Pimentel-Acosta.
Review and Editing: FN Morales-Serna, VH Caña-Bozada, D Toala-Franco, EJ Fájer-Ávila.

References

1. Taylor K, Rego Alvarez L. Regulatory drivers in the last 20 years towards the use of *in silico* techniques as replacements to animal testing for cosmetic-related substances. Computational Toxicology. 2020;13:100112. doi: 10.1016/j.comtox.2019.100112.
2. Kashkooli FM, Soltani M, Souri M, Meaney C, Kohandel M. Nexus between *in silico* and *in vivo* models to enhance clinical translation of nanomedicine. Nano Today. 2021;36:101057. doi: 10.1016/j.nantod.2020.101057.
3. Shen D, Chang HR, Chen Z, et al. Loss of annexin A1 expression in human breast cancer detected by multiple high-throughput analyses. Biochemical and Biophysical Research Communications. 2004;326(1):218—227. doi: 10.1016/j.bbrc.2004.10.214.

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

4. Wang XS, Zhang Z, Wang HC, et al. Rapid Identification of UCA1 as a very sensitive and specific unique marker for human bladder carcinoma. Clinical Cancer Research. 2006;12(16):4851—4858. doi: 10.1158/1078-0432.CCR-06-0134.
5. Murray D, Doran P, MacMathuna P, Moss AC. *In silico* gene expression analysis – an overview. Molecular Cancer. 2007;6(1):50. doi: 10.1186/1476-4598-6-50.
6. Diep B, Barretto C, Portmann AC, et al. Salmonella serotyping; comparison of the traditional method to a microarray-based method and an *in silico* platform using whole genome sequencing data. Frontiers in Microbiology. 2019;10:2554. doi: 10.3389/fmicb.2019.02554.
7. Ogawa K. Diseases of cultured marine fishes caused by Platyhelminthes (Monogenea, Digenea, Cestoda). Parasitology. 2015;142(1):178—195. doi: 10.1017/S0031182014000808.
8. Zhi T, Xu X, Chen J, et al. Expression of immune-related genes of Nile tilapia *Oreochromis niloticus* after *Gyrodactylus cichlidarum* and *Cichlidogyrus sclerosus* infections demonstrating immunosupression in coinfection. Fish & Shellfish Immunology. 2018;80:397—404. doi: 10.1016/j.fsi.2018.05.060.
9. Igeh PC, Avenant–Oldewage A. Pathological effects of *Cichlidogyrus philander* Douëllou, 1993 (Monogenea, Ancyrocephalidae) on the gills of *Pseudocrenilabrus philander* (Weber, 1897) (Cichlidae). Journal of Fish Diseases. 2020;43(2):177—184. doi: 10.1111/jfd.13121.
10. Prabu E, Rajagopalsamy CBT, Ahilan B, Jeevagan IJMA, Renuhadevi M. Tilapia – an excellent candidate species for world aquaculture: a review. ARRB. 2019:1—14. doi: 10.9734/arrb/2019/v31i330052.
11. Martínez-Cordeo FJ, Delgadillo TS, Sánchez-Zazueta E, Cai J. Tilapia aquaculture in Mexico - Assessment with a focus on social and economic performance. Vol. 12019. FAO; 2021. doi: 10.4060/cb3290en.
12. Paredes-Trujillo A, Velázquez-Abunader I, Torres-Irineo E, Romero D, Vidal-Martínez VM. Geographical distribution of protozoan and metazoan parasites of farmed Nile tilapia *Oreochromis niloticus* (L.) (Perciformes: Cichlidae) in Yucatán, México. Parasites Vectors. 2016;9(1):66. doi: 10.1186/s13071-016-1332-9.
13. Paredes-Trujillo A, Mendoza-Carranza M, Río-Rodriguez RED, Cerqueda-García D. Comparative assessment of metazoans infestation of Nile tilapia (*Oreochromis niloticus*) (L.) (Perciformes: Cichlidae) in floating cages and ponds from Chiapas, Mexico. Veterinary Parasitology: Regional Studies and Reports. 2022;34:100757. doi: 10.1016/j.vprsr.2022.100757.
14. Morales-Serna FN, Medina-Guerrero RM, Pimentel-Acosta C, Ramírez-Tirado JH, Fajer-Ávila EJ. Parasite infections in farmed Nile tilapia *Oreochromis niloticus* in Sinaloa, Mexico. Comparative Parasitology. 2018;85(2):212—216. doi: 10.1654/1525-2647-85.2.212.
15. Osuna-Cabanillas JM, Medina-Guerrero RM, Camacho-Zepeda S, Morales-Serna FN, Fajer-Ávila EJ. Prevalencia e intensidad de tricodínidos y monogeneos en tilapia cultivada en el suroeste de México. Ecosistemas y Recursos Agropecuarios. 2022;9(2). doi: 10.19136/era.a9n2.3290.
16. Aguirre-Fey D, Benítez-Villa GE, Pérez-Ponce de León G, Rubio-Godoy M. Population dynamics of *Cichlidogyrus* spp. and *Scutogyrus* sp. (Monogenea) infecting farmed tilapia in Veracruz, México. Aquaculture. 2015;443:11—15. doi: 10.1016/j.aquaculture.2015.03.004.

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

17. Valladão GMR, Gallani SU, Pilarski F. Phytotherapy as an alternative for treating fish disease. Journal of Veterinary Pharmacology and Therapeutics. 2015;38(5):417–428. doi: 10.1111/jvp.12202.
18. Matoušková P, Vokřál I, Lamka J, Skálová L. The role of xenobiotic-metabolizing enzymes in anthelmintic deactivation and resistance in helminths. Trends in Parasitology. 2016;32(6):481–491. doi: 10.1016/j.pt.2016.02.004.
19. Abaza S. Recent advances in identification of potential drug targets and development of novel drugs in parasitic diseases. Part II: Parasite targets. Parasitologists United Journal. 2022;15(1):22–38. doi: 10.21608/PUJ.2022.129311.1160.
20. Mate L, Ballent M, Cantón C, et al. ABC-transporter gene expression in ivermectin-susceptible and resistant *Haemonchus contortus* isolates. Veterinary Parasitology. 2022;302:109647. doi: 10.1016/j.vetpar.2022.109647.
21. Langeland A, Jetter H, O'Halloran DM. The diversity of ABC transporter genes across the Phylum Nematoda. Parasitology International. 2021;83:102357. doi: 10.1016/j.parint.2021.102357.
22. Caña-Bozada V, Morales-Serna FN, Fajer-Ávila EJ, Llera-Herrera R. *De novo* transcriptome assembly and identification of G-Protein-Coupled-Receptors (GPCRs) in two species of monogenean parasites of fish. Parasite. 2022;29:51. doi: 10.1051/parasite/2022052.
23. Caña-Bozada V, Morales-Serna FN, García-Gasca A, Llera-Herrera R, Fajer-Ávila EJ. Genome-wide identification of ABC transporters in monogeneans. Molecular and Biochemical Parasitology. 2019;234:111234. doi: 10.1016/j.molbiopara.2019.111234.
24. Pimentel-Acosta CA, Ramírez-Salcedo J, Morales-Serna FN, et al. Molecular effects of silver nanoparticles on monogenean parasites: Lessons from *Caenorhabditis elegans*. International Journal of Molecular Sciences. 2020;21(16):5889. doi: 10.3390/ijms21165889.
25. Altschul S. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research. 1997;25(17):3389–3402. doi: 10.1093/nar/25.17.3389.
26. Finn RD, Coggill P, Eberhardt RY, et al. The Pfam protein families database: towards a more sustainable future. Nucleic Acids Res. 2016;44(D1):D279–D285. doi: 10.1093/nar/gkv1344.
27. Sheps JA, Ralph S, Zhao Z, Baillie DL, Ling V. The ABC transporter gene family of *Caenorhabditis elegans* has implications for the evolutionary dynamics of multidrug resistance in eukaryotes. Genome Biology. 2004;5(3):R15. doi: 10.1186/gb-2004-5-3-r15.
28. Grabherr MG, Haas BJ, Yassour M, et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnology. 2011;29(7):644–652. doi: 10.1038/nbt.1883.
29. De Castro E, Sigrist CJA, Gattiker A, et al. ScanProsite: detection of PROSITE signature matches and ProRule-associated functional and structural residues in proteins. Nucleic Acids Research. 2006;34(Web Server):W362–W365. doi: 10.1093/nar/gkl124.
30. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research. 2004;32(5):1792–1797. doi: 10.1093/nar/gkh340.

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

31. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics. 2009;25(15):1972–1973. doi: 10.1093/bioinformatics/btp348.
32. Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods. 2017;14(6):587–589. doi: 10.1038/nmeth.4285.
33. Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution. 2015;32(1):268–274. doi: 10.1093/molbev/msu300.
34. Rambaut, A. Figtree 1.4.4. http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 31 July 2020).
35. Cheadle C, Vawter MP, Freed WJ, Becker KG. Analysis of microarray data using z score transformation. The Journal of Molecular Diagnostics. 2003;5(2):73–81. doi: 10.1016/S1525-1578(10)60455-2.
36. Wurmbach E, Yuen T, Sealfon SC. Focused microarray analysis. Methods. 2003;31(4):306–316. doi: 10.1016/S1046-2023(03)00161-0.
37. Cvilink V, Lamka J, Skálová L. Xenobiotic metabolizing enzymes and metabolism of anthelminthics in helminths. Drug Metabolism Reviews. 2009;41(1):8–26. doi: 10.1080/03602530802602880.
38. McColl ER, Vassileva V, Piquette-Miller M. Drug Transporters: Efflux. In: Comprehensive Pharmacology. Amsterdam, Netherlands: Elsevier; 2022. pp. 608–626. doi: 10.1016/B978-0-12-820472-6.00054-2.
39. Sepúlveda–Crespo D, Reguera RM, Rojo–Vázquez F, Balaña–Fouce R, Martínez–Valladares M. Drug discovery technologies: *Caenorhabditis elegans* as a model for anthelmintic therapeutics. Medicinal Research Reviews. 2020;40(5):1715–1753. doi: 10.1002/med.21668.
40. Kruh GD, Belinsky MG. The MRP family of drug efflux pumps. Oncogene. 2003;22(47):7537–7552. doi: 10.1038/sj.onc.1206953.
41. Daood MJ, Ahdab-Barmada M, Watchko JF. Comparison of multidrug resistance protein-1 (MRP-1) and P-glycoprotein (PGP) expression in the developing human central nervous system: cellular and tissue localization. Pediatric Research. 2004;56(4):673–673. doi: 10.1203/00006450-200410000-00062.
42. Kerboeuf D. P-glycoprotein in helminths: function and perspectives for anthelmintic treatment and reversal of resistance. International Journal of Antimicrobial Agents. 2003;22(3):332–346. doi: 10.1016/S0924-8579(03)00221-8.
43. Gerhard AP, Krücken J, Heitlinger E, et al. The P-glycoprotein repertoire of the equine parasitic nematode *Parascaris univalens*. Scientific Reports. 2020;10(1):13586. doi: 10.1038/s41598-020-70529-6.
44. Martin F, Dube F, Karlsson Lindsjö O, et al. Transcriptional responses in *Parascaris univalens* after in vitro exposure to ivermectin, pyrantel citrate and thiabendazole. Parasites Vectors. 2020;13(1):342. doi: 10.1186/s13071-020-04212-0.
45. Williamson SM, Storey B, Howell S, Harper KM, Kaplan RM, Wolstenholme AJ. Candidate anthelmintic resistance-associated gene expression and sequence polymorphisms in a triple-resistant field isolate of *Haemonchus contortus*. Molecular and Biochemical Parasitology. 2011;180(2):99–105. doi: 10.1016/j.molbiopara.2011.09.003.

doi: 10.22201/fmvz.24486760e.2024.1273
Vol. 11 | 2024

46. Kaur J, Dey CS. Putative P-glycoprotein expression in arsenite-resistant *Leishmania donovani* down-regulated by verapamil. Biochemical and Biophysical Research Communications. 2000;271(3):615–619. doi: 10.1006/bbrc.2000.2680.
47. Wartenberg M, Ling FC, Schallenberg M, et al. Down-regulation of Intrinsic P-glycoprotein expression in multicellular prostate tumor spheroids by reactive oxygen species. Journal of Biological Chemistry. 2001;276(20):17420–17428. doi: 10.1074/jbc.M100141200.
48. Russel F, Koenderink J, Masereeuw R. Multidrug resistance protein 4 (MRP4/ABCC4): a versatile efflux transporter for drugs and signalling molecules. Trends in Pharmacological Sciences. 2008;29(4):200–207. doi: 10.1016/j.tips.2008.01.006.
49. Yan R, Urdaneta-Marquez L, Keller K, James CE, Davey MW, Prichard RK. The role of several ABC transporter genes in ivermectin resistance in *Caenorhabditis elegans*. Veterinary Parasitology. 2012;190(3–4):519–529. doi: 10.1016/j.vetpar.2012.06.038.
50. Raza A, Bagnall NH, Jabbar A, Kopp SR, Kotze AC. Increased expression of ATP binding cassette transporter genes following exposure of *Haemonchus contortus* larvae to a high concentration of monepantel in vitro. Parasites Vectors. 2016;9(1). Art no 522. doi: 10.1186/s13071-016-1806-9.
51. Raza A, Kopp SR, Bagnall NH, Jabbar A, Kotze AC. Effects of in vitro exposure to ivermectin and levamisole on the expression patterns of ABC transporters in *Haemonchus contortus* larvae. International Journal for Parasitology: Drugs and Drug Resistance. 2016;6(2):103–115. doi: 10.1016/j.ijpddr.2016.03.001.
52. James CE, Hudson AL, Davey MW. Drug resistance mechanisms in helminths: is it survival of the fittest? Trends in Parasitology. 2009;25(7):328–335. doi: 10.1016/j.pt.2009.04.004.