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AVALIAÇÃO DO PROTEOMA DE *Trichuris trichiura* COMO POTENCIAL FONTE DE MOLÉCULAS COM ATIVIDADE IMUNOMODULATÓRIA

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Tese apresentada ao Programa de Pós-Graduação em Biotecnologia, Instituto de Ciências da Saúde, Universidade Federal da Bahia como requisito para obtenção do título de Doutor em Biotecnologia.

Orientadora: Profa. Dra. Carina da Silva Pinheiro

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"É preciso imaginar Sísifo feliz" (Albert Camus)

SANTIAGO, Leonardo Freire. AVALIAÇÃO DO PROTEOMA DE *Trichuris trichiura* COMO POTENCIAL FONTE DE MOLÉCULAS COM ATIVIDADE IMUNOMODULATÓRIA. Orientadora: Carina da Silva Pinheiro. 102 f. 2023. Tese de Doutorado - Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador, 2023.

RESUMO

Helmintos transmitidos pelo solo (STHs), como *Trichuris trichiura*, representam um significativo desafio para a saúde global, afetando particularmente crianças e resultando em considerável morbidade. No entanto, a capacidade do *T. trichiura* em manipular as respostas imunes do hospedeiro oferece uma promissora via para identificar biomoléculas com potencial terapêutico em distúrbios inflamatórios, alérgicos e autoimunes. Este estudo marcaa primeira exploração abrangente do proteoma de vermes adultos de *T. trichiura*, machos e fêmeas, utilizando cromatografia líquida acoplada à espectrometria de massa em tandem (LC-MS/MS). A análise resultou na identificação de 810 proteínas do parasito. Ontologia genética (GO) foi empregada para avaliar o perfil de cada grupo (macho integral e somente as proteínas exclusivas, fêmea integral e somente as proteínas exclusivase proteínas compartilhadas entre os gêneros). Isso possibilitou a identificação de grupos proteicos com seus respectivos componentes, funções e processos, contribuindo para um melhor entendimento da biologia do parasito. Notavelmente, diversas proteínas com potencialimunomodulatório surgiram em ambos os sexos, incluindo o inibidor de protease do tipo Kunitz e a glutamato desidrogenase, apresentando promissoras aplicações terapêuticas. A proteína rc4299 produzida de forma recombinante demonstrou características imunomodulatórias, particularmente um aumento na secreção de IL-10, sugerindo seu potencial uso no tratamentode doenças autoimunes e alérgicas. Este estudo lança luz sobre as complexidades do proteomade *T. trichiura*, revelando promissores alvos terapêuticos e enfatizando a intrincada interaçãoentre o parasito e o sistema imune do hospedeiro.

Palavres-chave: Proteoma, *Trichuris trichiura*, Proteínas imunomoduladoras.

SANTIAGO, Leonardo Freire. EVALUATION OF THE PROTEOME OF *Trichuris trichiura* AS A POTENTIAL SOURCE OF MOLECULES WITH IMMUNOMODULATORY ACTIVITY. Thesis advisor: Carina da Silva Pinheiro. 2023.102 pp. Doctoral Thesis - Institute of Health Sciences, Federal University of Bahia, Salvador, 2023.

ABSTRACT

Soil-transmitted helminths (STHs), such as *Trichuris trichiura*, represent a significant global health challenge, particularly affecting children and leading to substantial morbidity. However, *T. trichiura's* ability to manipulate the host's immune responses offers a promisingavenue for identifying biomolecules with therapeutic potential in inflammatory, allergic, andautoimmune disorders. This study marks the inaugural comprehensive exploration of the proteome of adult male and female *T. trichiura* worms using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The analysis resulted in the identification of 810 parasite proteins. Genetic ontology was employed to assess the profile of each group (complete male, exclusive proteins, complete female, exclusive proteins, and proteins sharedbetween genders). This facilitated the identification of protein groups with their respective components, functions, and processes, contributing to a deeper comprehension of the parasite's biology. Notably, several proteins with immunomodulatory potential surfaced in both genders, including a Kunitz protease inhibitor and glutamate dehydrogenase, showcasingpromise for therapeutic applications. The recombinantly produced rc4299 protein demonstrated immunomodulatory characteristics, notably an increase in IL-10 secretion, suggesting potential use in treating autoimmune and allergic diseases. This study illuminatesthe intricacies of the *T. trichiura* proteome, uncovering promising therapeutic targets and emphasizing the intricate interplay between the parasite and the host's immune system.

Keywords: Proteome, *Trichuris trichiura*, Immunomodulatory proteins

LISTA DE ABREVIATURAS

AA	Amino acid (inglês); Aminoácido (português)
CD	Crohn's disease (inglês); doença de Crohn (português)
DALYs	Disability-adjusted life years (inglês); Anos de vida ajustados por incapacidade(português)
FBPA	Frutose bifosfato aldolase
GO	Gene ontology (inglês); Ontologia genética (português)
His	Histidine (inglês); histidina (português)
HSP70	Proteína de choque térmico 70
IgA	Immunoglobulin A (inglês); Imunoglobulina A (português)
IgE	Immunoglobulin E (inglês); Imunoglobulina E (português)
IgG	Immunoglobulin G (inglês); Imunoglobulina G (português)
IgG1	Imunoglobulina G1
IgG4	Imunoglobulina G4
IL – 4	Interleucina 4
IL – 5	Interleucina 5
IL – 9	Interleucina 9
IL – 10	Interleucina 10
IL – 12	Interleucina 12
IL – 13	Interleucina 13
IL – 25	Interleucina 25
IL – 33	Interleucina 33
INF-gamma(γ)	Interferon-gamma (português)
IPTG	Isopropyl β-D-1-thiogalactopyranoside (inglês); Isopropil-β-D-tiogalactopirosídeo(português)
kDa	Kilodalton
LAMP	Loop-mediated amplification (inglês); Amplificação mediada por loop (português)
LB	Luria-Bertani
LC-MS/MS	Cromatografia líquida acoplada à espectrometria de massa
Lys(K)	Lysine (inglês); Lisina (português)
MALDI-TOF	Espectrometria de massa com dessorção a laser/tempo de voo de ionizaçãoassistida por matriz

MS	Espectrometria de massa
MS2	Espectrometria de massa em tandem
MHC	Major histocompatibility complex (inglês); Complexo principal de histocompatibilidade (português)
MIFH	Fator inibidor da migração de macrófagos
mM	Milimolar
MS/MS	Espectrometria de massa em tandem
MW	Molecular weight (inglês); peso molecular (português)
NaP	Sodium phosphate (inglês); fosfato de sódio (português)
Nm	Nanômetro
nTreg	Células T reguladoras naturais
OD	Optical density(inglês); Densidade óptica (português)
OMS	Organização mundial da saúde
PAMP	Padrões moleculares associadas a patógenos
PBMCs	Células Mononucleares de Sangue Periférico
PCR	Polymerase chain reaction (inglês); Reação em cadeia da polimerase (português)
regDCs	Células dendríticas reguladoras
RI	Resistência à insulina
SCFAs	Ácidos graxos de cadeia curta SHT - Helmintos transmitidos pelo solo
TDS	Síndrome disentérica causada por *Trichuris*
TGF-β	Fator Transformador do Crescimento Beta
TH1	T helper 1 lymphocytes (inglês); Linfócitos T helper 1 (português)
TH2	T helper 2 lymphocytes (inglês); Linfócitos T helper 2 (português)
TLR	Receptores semelhantes à Toll
TLR2	Receptores semelhantes à Toll 2
TLR4	Receptores semelhantes à Toll 4
TSLP	Linfopoietina estromal tímica
TSO	Tratamento com Ovos de *Trichuris*
UC	Ulcerative colitis (inglês); colite ulcerativa (português)

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1 INTRODUÇÃO

Helmintos transmitidos pelo solo (HTS), também conhecidos como geohelmintos, ainda hoje são considerados um importante problema de saúde pública, especialmente em regiões mais pobres, onde fatores ambientais e sociais proporcionam um ambiente favorável à disseminação e persistência da infecção por esses parasitos (WHO, 2023). As infecções por geohelmintos estão entre as infecções parasitárias humanas mais comuns e afetam principalmente crianças de populações mais pobres e carentes de regiões nas quais o acesso à água, saneamento básico e higiene normalmente são limitados ou inadequados (CALDRER; URSINI; SANTUCCI; MOTTA *et al.*, 2022). Estimativas recentes indicam que cerca de um quarto da população mundial está em risco de infecção por geohelmintos,sendo que na América Latina e no Caribe aproximadamente 46 milhões de crianças vivem em áreas de alto risco de infecção (WRIGHT; WERKMAN; DUNN; ANDERSON, 2018). O parasito intestinal *Trichuris trichiura* é um dosmais frequentes geohelmintos, causador da tricuríase humana e responsável por aproximadamente 465milhões de infecções em todo o mundo (LOUKAS; MAIZELS; HOTEZ, 2021).

A tricuríase é uma doença de morbidade significativa; a gravidade da doença dependede fatores como idade, nutrição, infecções pré-existentes e carga parasitária. As manifestações clínicas da tricuríase podem variar desde formas leves ou assintomáticas, que causam desconforto ou dor abdominal, mal-estar geral e fraqueza, até formas sintomáticas que cursam com síndrome disentérica, gerando diarreia mucóide, sangramento e, em casos de elevada carga parasitária, prolapso retal no indivíduo infectado. Em crianças a infecção pode resultar em anemia por deficiência de ferro, retardo de crescimento e comprometimento cognitivo (AHMED, 2023). A ausência de uma vacina eficaz, associada à dificuldades no diagnóstico e relatos recentes de resistência à drogas anti-helmínticas demonstram que o controle dainfecção e da patologia causada por *T. trichiura* continua sendo um desafio à saúde pública e requer abordagens diferenciadas e mais eficientes (FISSIHA; KINDE, 2021; VAN DER REE; MUTAPI, 2015).

Parasitos helmintos como *T. trichiura* co-evoluíram com seus hospedeiros humanos, criando um ambiente imunomodulado favorável à sua sobrevivência. Possuem um complexo mecanismo de escape, onde mimetizam e manipulam o sistema imunológico do hospedeiro por meio de moléculas bioativas, principalmente proteínas, responsáveis por modular a resposta imune do parasitado a seu favor, adaptando a resposta ao parasitismo (MAIZELS;

SMITS; MCSORLEY, 2018; STEVENSON; VALANPARAMBIL; TAM, 2022). Apesar da resposta principal contra helmintos ser caracterizada por um perfil celular T helper tipo 2 (Th2), esses organismos conseguem persistir no hospedeiro por longos períodos em infecçõescrônicas, devido à produção de citocinas como a Interleucina 10 (IL-10) e o Fator Transformador do Crescimento Beta (TGF-β) (DARLAN; ROZI; YULFI, 2021; DIGE; RASMUSSEN; NEJSUM; HAGEMANN-MADSEN *et al.*, 2017). Essa habilidade de regulação do sistema imune pelos parasitos tem-se mostrado promissora, principalmente em doenças inflamatórias, alérgicas e autoimunes, onde a distração ou a modulação da imunidade deprimem suficientemente a reatividade imunológica, gerando redução da sintomatologia nessas desordens clínicas (DE ANDRADE; CARNEIRO; CERQUEIRA; FONSECA *et al.*, 2019; DING; LIU; BAI; WANG *et al.*, 2020).

A ingestão de ovos viáveis de *T. trichiura* para tratamento de pacientes com colite ulcerativa, uma condição inflamatória intestinal, já foi avaliada. Como resultado verificou-se que a colonização do intestino pelo parasito tem a capacidade de reduzir significativamente os sintomas da colite ulcerativa sintomática (BROADHURST; LEUNG; KASHYAP; MCCUNE *et al.*, 2010). Embora esse achado tenha relevância significante e aponte que a infecção por *T. trichiura* seja útil para aliviar os sintomas de doenças inflamatórias, o tratamento com ovos do parasito e as condições ideais para que se tenha qualquer benefício clínico ainda é incerto (BROADHURST, 2012). Além disso, o tratamentoalopático tradicional requer que a molécula utilizada como fármaco em medicamento biológico seja pura, preferencialmente molécula única, funcional e segura (FLOWER, 2022).

Abordagens proteômicas têm sido utilizadas para melhor compreensão da interação parasito-hospedeiro e na identificação de biomoléculas em parasitos que possam ser utilizadas como antígenos em diagnósticos, em alvos para fármacos e em medicamentos imunomoduladores (ROBINSON; CWIKLINSKI, 2021). Estudos prévios conduzidos pelo nosso grupo de pesquisa se concentraram na análise das frações protéicas do produto somático secretado/excretado pelo *T. trichiura*. Ao estimular as células mononucleares de sangue periférico (PBMCs) com essas frações observou-se um efeito imunomodulador significativo (SANTOS; GALLO; SILVA; FIGUEIREDO *et al.*, 2013). Além disso, o proteoma do extratode ovo de *T. trichiura* foi recentemente descrito e apontou diversas moléculas com potencial imunomodulador (CRUZ; MARCILLA; KELLY; VANDENPLAS *et al.*, 2021). Deste modo,o proteoma integral de *T. trichiura* adulto pode oferecer uma visão abrangente dos

mecanismos de interação parasito-hospedeiro e identificar biomoléculas candidatas com potencial para utilização como drogas imunomoduladoras.

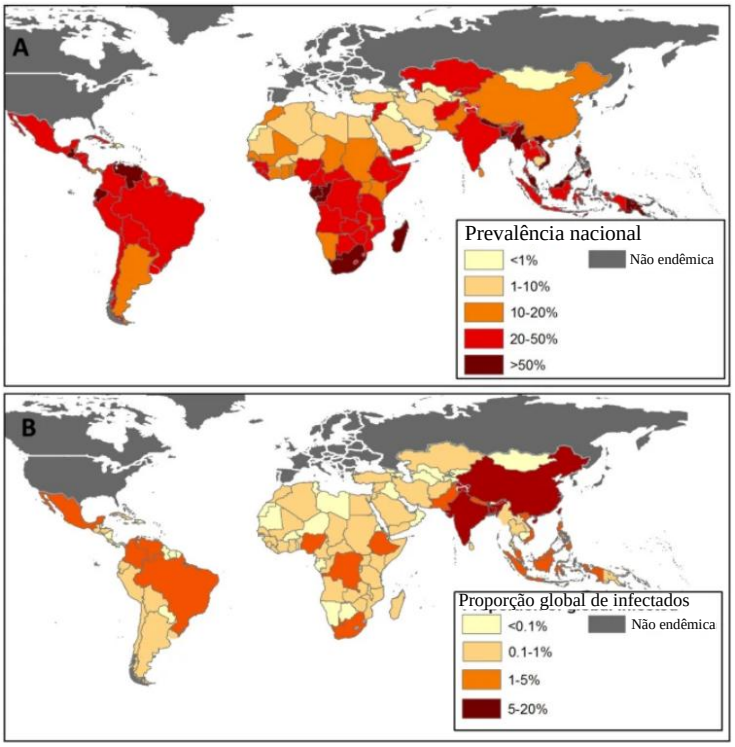
Neste estudo, empregamos uma abordagem proteômica utilizando cromatografia líquida acoplada à espectrometria de massa em tandem (LC-MS/MS) e aplicações de bioinformática para identificar, analisar e caracterizar o proteoma de parasitos adultos machos e fêmeas de *T. trichiura*. Além disso, uma molécula não caracterizada do parasito, localizada nesse proteoma, foi sintetizada e avaliada quanto ao seu potencial imunomoduladorem PBMCs.

2 FUNDAMENTAÇÃO TEÓRICA

2.1 EPIDEMIOLOGIA DAS GEOHELMINTÍASES

A maioria das infecções por geohelmintos é atribuída às espécies *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* e *Ancylostoma duodenale*. Juntos são responsáveis por grande parte do impacto global associado às infecções por helmintos e continuam a representar desafios significativos de saúde pública em todo o mundo(MOGAJI; JOHNSON; ADIGUN; ADEKUNLE *et al.*, 2022). Classificadas pela Organização Mundial de Saúde (OMS) como Doenças Tropicais Negligenciadas (DTNs), as geohelmintíases estão entre as doenças parasitárias mais comuns que atingem diretamente a população mais carente de países situados em áreas tropicais e subtropicais (Figura 1). São responsáveis por uma carga global estimada de doença de mais de 3,3 milhões de anos de vida ajustados por incapacidade (DALYs) (SCHLUTH; STANDLEY; BANSAL; CARLSON, 2023).

Figura 1 - Distribuição da infecção por geohelmintos..



(A) Prevalência nacional estimada por modelos de geoestatística. **(B)** Proporção da população mundial infectada (1,5 bilhão). Fonte: Adaptado de (PULLAN; SMITH; JASRASARIA; BROOKER, 2014).

Grande parte dessas infecções ocorre na Ásia, com destaque para China e Índia, onde 21% e 18% dos indivíduos estão coinfectados por mais de um geohelminto, respectivamente. Em contraste, os três países mais populosos da Áfricaapresentaram apenas 8% do total de infecções por esses parasitos (PULLAN; SMITH; JASRASARIA; BROOKER, 2014)

Isoladamente, o helminto *T. trichiura* é responsável pela infecção de aproximadamente 465 milhões de pessoas, com uma carga global de doença estimada em 640.000 DALYs. Em 45 países a taxa de prevalência por esse parasito foi > 20%, sendo preconizado a quimioprofilaxia preventiva como medida terapêutica e de controle (CRUZ; MARCILLA; KELLY; VANDENPLAS *et al.*, 2021; PULLAN; SMITH; JASRASARIA; BROOKER, 2014)

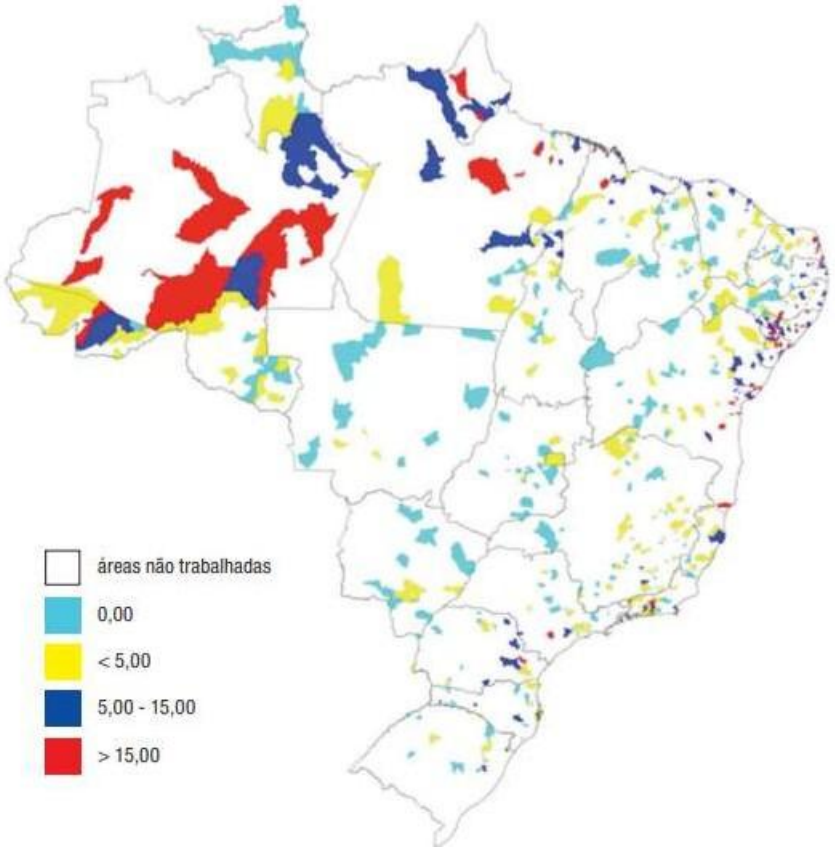
O Brasil possui prevalência estimada de 20 a 50% para os geohelmintos (PULLAN; SMITH; JASRASARIA; BROOKER, 2014). Como os dados provenientes do diagnóstico desses parasitos não são de notificação obrigatória pelos centros de saúde, os dados epidemiológicos são colhidos em inquéritos independentes e em levantamentos em conjunto com outras patologias. Nas regiões endêmicas para esquistossomose, os serviços de atenção básica à saúde detectou, no período de 2003 a 2012,um total de 88.850 casos positivos para *T. trichiuria*, 4,4% de positividade média (BRASIL, 2014).

Uma nova avaliação foi realizada em 2018, onde foi possível estimar que *T. trichiura* teve total de positividade geral no país de 5,41%, estando presente em 352 (66,56%) dos 521 municípios avaliados (Figura 2). A região norte do país é a mais afetada, com presença do parasito em 75,61% dos municípios testados, seguido pela região nordeste, onde 75,34% dos municípios testados identificaram o parasito. Norte e nordeste também lideram o percentual de positividade no país, com 15,8% e 5,93%, respectivamente, sendo que as maiores proporções de positivos para *T.trichiura* estão nos estados do Amazonas (21,79%), Pará (20,69%), Sergipe (16,99%) e Alagoas (15,04%). No país, indivíduos do gênero masculino (5,85%) são os mais afetados em relação ao gênero feminino (4,96%) (KATZ, N. 2018).

T. trichiura é o segundo parasito intestinal mais frequente em colegiais da cidade de Olinda, em Pernambuco (AGUIAR-SANTOS; MEDEIROS; BONFIM; ROCHA *et al.*, 2013). No município de Ilha das Flores (Sergipe), a prevalência encontrada foi de 54,8% (ROLLEMBERG; SANTOS; SILVA; SOUZA *et al.*, 2011; ROLLEMBERG; SILVA;

ROLLEMBERG; AMORIM *et al.*, 2015). Em Salvador (Bahia), após implantação do programa de saneamento Bahia azul, a prevalência teve redução de 18% para 5% (BARRETO;GENSER; STRINA; TEIXEIRA *et al.*, 2010)

Figura 2 – Distribuição da tricuríase no Brasil.



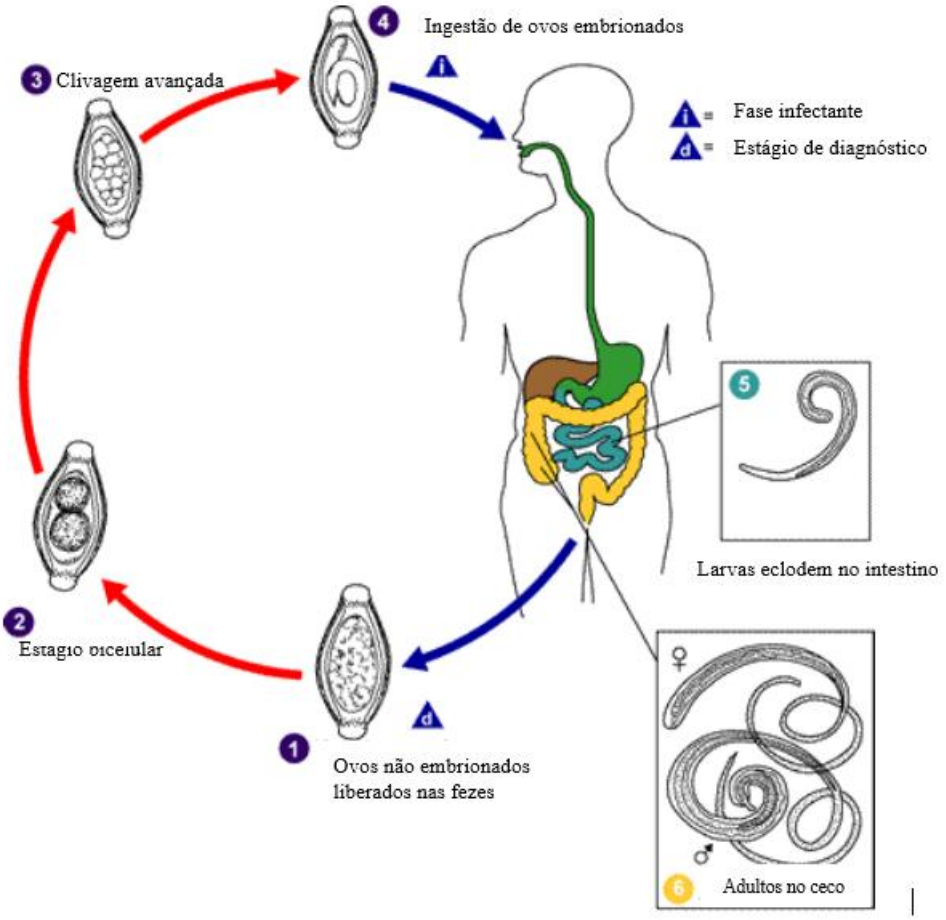
Fonte: KATZ, 2018.

2.2 TRICURÍASE

Tricuríase é uma infecção intestinal cujo agente etiológico é o parasito *T. trichiura*, sendo os humanos os principais hospedeiros e transmissores do parasito. A infecção ocorre por ingestão de alimentos ou água contaminados com ovos embrionados (infectantes) do parasito (Figura 3). Devido a estímulos da microbiota bacteriana no cólon, os ovos de *T. trichiura* eclodem e liberam as larvas L1, larvas de primeiro estágio que penetram nas células epiteliais

que revestem as criptas de Lieberkühn e, nesse local, crescem e mudam a L2, L3, L4, L5 até o estágio adulto. Machos e fêmeas sexualmente maduros acasalam e a fêmea libera, junto às fezes do indivíduo infectado, os ovos não embrionados. No solo, em condições adequadas de temperatura e umidade, os ovos se tornam infectantes (figura 2B) (ELSE; KEISER; HOLLAND; GRENCIS *et al.*, 2020; SARGSIAN; CHEN; LEE; ROBERTSON *et al.*, 2022).

Figura 3 - Ciclo biológico do *Trichuris trichiura.*



Fonte: Adaptada de Centers for deseases control and prevention, disponível em: http://www.cdc.gov/parasites/whipworm/biology.html.

A infecção inicial é normalmente assintomática. Porém, o parasito pode promover intensa colonização do cólon intestinal, principalmente em crianças, e gerar uma síndrome conhecida como SDT (síndrome disentérica causada por *Trichuris*), causando diarreia muco sanguinolenta, sangramento retal, anemia por deficiência de ferro, prolapso retal e hipocratismo

digital (AHMED, 2023; OWADA; NIELSEN; LAU; CLEMENTS *et al.*, 2017). Em adultos, a elevada infecção pode levar a quadros de SDT ou colite crônica com quadrosclínicos similares à outras doenças intestinais como, por exemplo, doença de Crohn e colite ulcerativa (BIANUCCI; TORRES; SANTIAGO; FERREIRA *et al.*, 2015).

O tratamento da tricuríase é baseado na administração de medicamentos anti-helmínticos como os benzimidazóis (albendazol ou mebendazol). Campanhas internacionais para o tratamento e quimioprofilaxia com o uso dessas drogas têm reduzido significantementeo número de indivíduos infectados; entretanto, reinfecções e casos de resistência são frequentemente relatados (OLLIARO; VAILLANT; DIAWARA; SPEICH *et al.*, 2022; PRIETO-PEREZ; PEREZ-TANOIRA; CABELLO-UBEDA; PETKOVA-SAIZ *et al.*, 2016). Ensaios clínicos têm demonstrado resultados promissores na associação quimioterápica, como albendazol e mebendazol, albendazol e pamoato de oxantel ou albendazol e ivermectina (MOSER; SCHINDLER; KEISER, 2019; PALMEIRIM; HURLIMANN; KNOPP; SPEICH *et al.*, 2018). Essas associações têm elevado as taxas de cura, reduzindo a quantidade de ovos eliminados e, consequentemente, as taxas de reinfecção na população analisada (SPEICH; MOSER; ALI; AME *et al.*, 2016).

Métodos de diagnóstico precisos são considerados um dos pilares para o controle da tricuríase, uma vez que possibilitam uma compreensão detalhada da epidemiologia da doença e permitem um acompanhamento mais eficaz dos pacientes infectados. Além disso, contribuem para a melhoria na implementação, planejamento e avaliação dos programas de desparasitação e vigilância em saúde (MISWAN; SINGHAM; OTHMAN, 2022).

Os métodos tradicionais comumente utilizados para detecção da infecção por esses vermes, como, por exemplo: coproscopia, Kato-Katz, Éter-concentrado e Kit FLOTAC, requerem condições específicas (fêmeas em período de oviposição, presença dos ovos na luz intestinal e nas fezes colhidas). Dessa forma, identificam apenas os ovos eliminados pelas fêmeas e não conseguem detectar o parasito em todas suas fases evolutivas no hospedeiro. Podendo subestimar, gerar falso negativo ou ainda grande variação entre os testes (KHURANA; SINGH; MEWARA, 2021).

Atualmente, o diagnóstico da tricuríase é realizado por meio de métodos parasitológicos diretos como o exame de sedimentação espontânea e o método de Kato-Katz e, incidentalmente, por visualização do parasito em colonoscopia (ISHIZAKI; KAWASHIMA; GUNJI; ONIZAWA *et al.*, 2022).

Abordagens moleculares são cada vez mais utilizadas no monitoramento e vigilância,

sendo úteis também em locais de baixa endemicidade (ZENDEJAS-HEREDIA; COLELLA; HII; TRAUB, 2021). Diagnósticos moleculares mais sensíveis, como a reação em cadeia da polimerase (PCR), PCR quantitativo (qPCR) (FISSIHA; KINDE, 2021), ensaio de amplificação mediada por loop (LAMP) (RASHWAN; DIAWARA; SCOTT; PRICHARD, 2017) e utilização de sistemas de análise de imagens para identificação e quantificação de ovos de helmintos, com potencial adaptação para smartphones, são tendências promissoras e já vêm sendo utilizadas em alguns trabalhos com boas perspectivas (KHURANA; SINGH; MEWARA, 2021). Mesmo com as vantagens de aumento na sensibilidade e especificidade, rapidez e segurança dos resultados, os métodos moleculares ainda são caros e com necessidadede altos investimentos. Deste modo, a tricuríase segue com a necessidade de desenvolvimento de metodologias eficientes e de fácil acesso para detecção e acompanhamento da doença.

2.3 RESPOSTA IMUNE AOS HELMINTOS

Ao longo de sua jornada no hospedeiro, o parasito passa por processos de desenvolvimento progressivo, migrando por diferentes sistemas e órgãos do indivíduo infectado até atingir a maturidade como vermes adultos. Essas transições de desenvolvimentoe migração ocorrem ao longo de um período variado, dependendo do parasito e da resposta imune do hospedeiro. Nesse contexto, as respostas imunitárias são frequentemente reguladas de maneira distinta, influenciadas pelo tecido em que o parasito tem fixação final, pelo tempo de vida do próprio parasito e por características do indivíduo infectado (GAZZINELLI-GUIMARAES; NUTMAN, 2018).

A resposta imune contra helmintos é predominantemente mediada pelos linfócitos T helper 2 (Th2) que se caracterizam por secretar interleucinas (IL) como IL-4, IL-5, IL-9 e IL-13, ativando eosinófilos, mastócitos, macrófagos e estimulando a produção de anticorpos IgE e IgG1. Essa resposta, que ocorre principalmente durante a migração larval, envolve alarminas como a linfopoietina estromal tímica (TSLP), IL-25 e IL-33 produzidas pelas células epiteliais intestinais e células TufT, que promovem e amplificam a imunidade tipo 2 através da ação das células linfoides inatas do grupo dois (ILC2s) (LOKE; LEE; OYESOLA, 2022; SMITH; LOSER; VARYANI; HARCUS *et al.*, 2018; YAP; GAUSE, 2018). O que causa hiperplasia de células caliciformes, muco em excesso, eosinofilia e macrófagos

alternativamente ativados M2. Produzindo deste modo mais IL-4 e IL-13, modificando células epiteliais e estromais para eliminar o parasito, fortalecer a barreira mucosa e promover a contração dos músculos intestinais para expulsar os helmintos (LOKE; LEE; OYESOLA, 2022; PRODJINOTHO; GRES; HENKEL; LACORCIA *et al.*, 2022). As reações iniciais e agudas dos helmintos muitas vezes se assemelham à respostas alérgicas (GAZZINELLI-GUIMARAES; NUTMAN, 2018).

A resposta imune Th2 é composta por três características clássicas: inflamação, reparo de lesões e resistência a helmintos (RAJASEKARAN; ANURADHA; BETHUNAICKAN, 2017). A degranulação de mastócitos, induzida pela IgE, determina mudanças na fisiologia intestinal, assim como na estrutura do epitélio intestinal. Essas mudanças ocorrem pela estimulação da secreção de fluidos, eletrólitos e muco, assim como acontração do músculo liso, aumento na permeabilidade epitelial e vascular e o recrutamentode eosinófilos, mastócitos e linfócitos aos locais de inflamação (YAP; GAUSE, 2018)

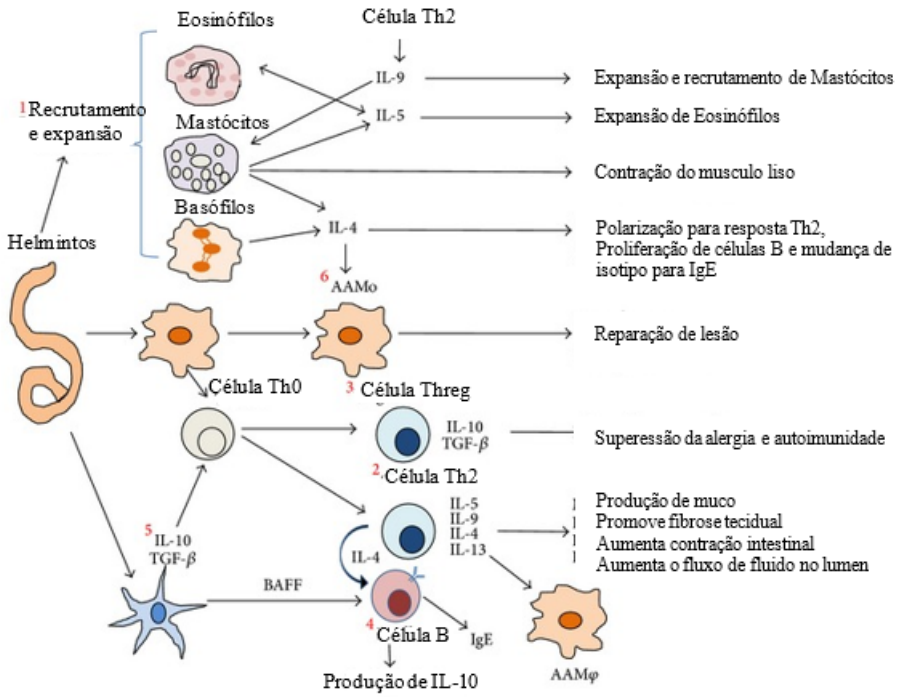
Um dos principais mecanismos envolvidos na eliminação dos parasitos é a citotoxicidade não fagocítica ocasionada por eosinófilos, macrófagos ou plaquetas; participam também deste tipo de citotoxicidade os anticorpos IgE, IgG ou IgA que recobrem a superfície do parasito, em seguida as células efetoras, através dos receptores específicos para cada um dos tipos de anticorpos (receptores Fc), promovem a lise do parasito, liberando diferentes mediadores inflamatórios e tóxicos como proteína catiônica eosinofílica (ECP), neurotoxina derivada de eosinófilos (EDN) e espécies reativas de nitrogênio (Figura 4) (LU; SUSCOVICH; FORTUNE; ALTER, 2018).

Nos últimos anos, a participação da resposta imune inata nas infecções parasitárias tem sido evidenciada. Nela, os receptores semelhantes à Toll (TLR2 e TLR4) e outros (receptores de Lectinas e outros TLRs) reconhecem os padrões moleculares associados a patógenos (PAMPs) e estimulam as células dendríticas a iniciar uma resposta Th2 (RAJASEKARAN; ANURADHA; BETHUNAICKAN, 2017).

Nas infecções crônicas, os helmintos estimulam uma resposta tipo Th2-modificada que permite a sobrevida do parasito e, em alguns casos, protege o indivíduo infectado contra processos inflamatórios e distúrbios imunológicos, como alergia, autoimunidade e síndrome metabólica (MAIZELS, 2020). Essa resposta resulta em uma modulação significativa via T helper 1 (Th1), com IL-2 e interferon-gama (IFN-γ) induzindo a expansão de células T

reguladoras naturais (nTreg), células dendríticas reguladoras (regDCs) e monócitos como fontes de IL-10 (GAZZINELLI-GUIMARAES; NUTMAN, 2018). Além disso, IL-4 e IL- 13 também estão envolvidas na ativação alternativa de macrófagos, que passam a produzir preferencialmente IL-10 (Figura 4) (ROLOT; DEWALS, 2018). A infecção crônica por helmintos também altera a composição das comunidades bacterianas intestinais, levando a um aumento nos ácidos graxos de cadeia curta (SCFAs) derivados de microorganismos, que também ativam e promovem a expansão das células Treg e, consequentemente, mais IL-10 (GAZZINELLI-GUIMARAES; NUTMAN, 2018).

Figura 4 - Principais eventos envolvidos na resposta do sistema imune frente a infecção por helmintos e mecanismos de escape utilizados por esses parasitos.



Fonte: Adaptado de: (SALAZAR-CASTANON; LEGORRETA-HERRERA; RODRIGUEZ-SOSA, 2014)

A resposta imune aos parasitos, influenciada por sua capacidade de evadir às defesas dos hospedeiros, leva a uma imunomodulação que permite sua sobrevida em um ambiente com pouca inflamação. Este processo é inespecífico e controla também a resposta a alérgenos e a estímulos que produzem as doenças autoimunes. Muitos estudos da associação entre as infecções helmínticas e a asma têm resultados contraditórios: alguns estudos mostraram que as infecções podem atuar como um fator protetor, outros mostram que podemser um fator de risco

para as doenças alérgicas (ARRAIS; MARICOTO; COOPER; GAMA *et al.*, 2020; MAIZELS, 2020).

2.4 MOLÉCULAS DE TRICURÍDEOS COM POTENCIAL FONTE MUNOMODULADORAS

Pesquisas recentes com espécies pertencentes ao gênero *Trichuris* demonstraram o potencial da utilização de diversas moléculas oriundas desses parasitos para uso em terapêutica de doenças inflamatórias (WILLIAMS; KLAVER; LAAN; RAMSAY *et al.*, 2017), bem como para imunoprofilaxia (WAINWRIGHT; SHEARS, 2022; WEI; HEGDE; YANAMANDRA; O'HARA *et al.*, 2022) .

Pacientes com doenças inflamatórias intestinais, como doença de Crohn e colite ulcerativa, obtiveram benefício terapêutico com infecções controladas com *Trichuris suis*, que causa infecção autolimitante em humanos (SUMMERS et al., 2005). Estudos clínicos de fases 1 e 2 demonstraram que o tratamento com ovos de *Trichuris suis* é seguro e bem tolerado em pacientes com esclerose múltipla, embora apresentem apenas uma eficácia clínica modesta (YORDANOVA; EBNER; SCHULZ; STEINFELDER *et al.*, 2021). Vários estudos indicam que a ausência de infecções por helmintos intestinais estáassociada a uma maior probabilidade de aparecimento de doenças inflamatórias intestinais (AXELRAD; CADWELL; COLOMBEL; SHAH, 2021), suscetibilidade a outras doenças imunomediadas (DOUGLAS; OYESOLA; COOPER; POSEY *et al.*, 2021; IANIRO; IORIO; PORCARI; MASUCCI *et al.*, 2022; WEINSTOCK; ELLIOTT, 2014), predisposição à síndrome metabólica (SANYA; WEBB; ZZIWA; KIZINDO *et al.*, 2020; TAHAPARY; DE RUITER; MARTIN; BRIENEN *et al.*, 2017) e predisposição para diabetes mellitus tipo 2 por aumento da resistência à insulina (ATAGOZLI; ELLIOTT; INCE, 2023; RAJAMANICKAM; MUNISANKAR; BHOOTRA; DOLLA *et al.*, 2019; TAHAPARY; DE RUITER; MARTIN; BRIENEN *et al.*, 2017). Produtos excretados/secretados de *T. suis* têm potencial de suprimir a produção de citocinas pró-inflamatórias em células epiteliais intestinais (HIEMSTRA; KLAVER; VRIJLAND; KRINGEL *et al.*, 2014). Além disso, a administração de proteínas do produto excretado/secretado da fase larval (L1) de *T. suis in vivo,* durante a fase de sensibilização alergênica, foi suficiente para suprimir a hiperreatividade das vias aéreas, o infiltrado inflamatório brônquico e a produção de IgE específica para alérgenos em modelo murino (EBNER; HEPWORTH; RAUSCH; JANEK *et al.*, 2014).

O *T. muris*, um tricurídeo que infecta camundongos, é usado como modelo de estudo para analisar as interações entre parasito e hospedeiro. Recentemente foi identificado que o secretoma de *T. muris* adultos é dominado por uma única proteína, com uma cauda de policisteína e histidina, conhecida como P43. Mais de 90% das proteínas totais secretadas foi composta por P43, que demonstrou capacidade de suprimir a função da IL-13, tanto *in vitro* como in vivo. Além disso, o homólogo de P43 em *T. trichiura* também apresentaafinidade pela IL-13 humana e é capaz de inibir sua função *in vitro* (SANTOS; GALLO; SILVA; FIGUEIREDO *et al.*, 2013).

Em estudo realizado por nosso grupo foi possível avaliar que as frações proteicas provenientes do extrato somático de *T. trichiura* apresentam importante função imunomoduladora em PBMCs de doadores saudáveis, com produção de IL-10 e TNF-α por PBMCs estimulados com diferentes frações proteicas do verme adulto (SANTOS; GALLO; SILVA; FIGUEIREDO *et al.*, 2013). Além disso foram identificadas por LC-MS/MS diversas proteínas do estrato somático do parasito, incluindo o homólogo do fator inibidor da migraçãode macrófagos (MIFH), a frutose bifosfato aldolase (FBPA) e a proteína de choque térmico 70 (HSP70), entre outras proteínas essenciais para o ciclo de vida do parasito e que possuem propriedades imunoreguladoras (AGUIAR-SANTOS; MEDEIROS; BONFIM; ROCHA *et al.*, 2013; PIROVICH; DA'DARA; SKELLY, 2021).

O proteoma do extrato do ovo de *T. trichiura* também revelou potenciais alvos imunomoduladores. Dentre as proteínas potencialmente imunoativas presentes no extrato, destacam-se a vitelogenina N, a isoforma 2 da proteína de cauda de policisteína e histidina (PCHTP-2), a proteína de choque térmico 70 (HSP70), endolase e a gliceraldeído-3-fosfato desidrogenase (CRUZ; MARCILLA; KELLY; VANDENPLAS *et al.*,2021).

As informações disponíveis indicam que o parasito *T. trichiura* pode ser uma fonte promissora de moléculas imunomoduladoras, com potencial aplicação terapêutica em doenças alérgicas e inflamatórias. Isso se deve ao perfil imunorreativo de diversas moléculasencontradas nos vermes dessa família. Essa perspectiva abre caminho para o desenvolvimentode moléculas imunoterapêuticas que podem ser utilizadas em um futuro próximo.

3 JUSTIFICATIVA E HIPÓTESE

T. trichiura, assim como outros helmintos, coevoluiu com os humanos. Diversos trabalhos com coprólitos (fezes fossilizadas) e corpos mumificados demonstraram a presença de ovos desse parasito nesses achados arqueológicos (BIANUCCI; TORRES; SANTIAGO; FERREIRA *et al.*, 2015; NERLICH; EGARTER VIGL; FLECKINGER; TAUBER *et al.*, 2021). Diversas estratégias de mimetismo molecular e fuga foram desenvolvidas ao longo do tempo pelo *T. trichiura* para burlar e escarpar do sistema imune de seu hospedeiro humano (ELSE; KEISER; HOLLAND; GRENCIS *et al.*, 2020; LOUKAS; MAIZELS; HOTEZ, 2021). Estratégias estas bem sucedidas, pois até hoje o parasito é um dos helmintos transmitidos pelo solo de maior frequência em todo mundo.

A avaliação do proteoma de *T. trichiura* gera uma oportunidade única de entendimento da biologia do parasito e a possibilidade de localizar moléculas proteicas presentes em seu proteoma que possam ser úteis na modulação do sistema imune e que possam auxiliar no tratamento de doenças alérgicas inflamatórias e autoimunes. Essa hipótese ganha mais suporte considerando que, como já mencionado anteriormente, tanto o proteoma dos ovos do parasito quanto seus produtos excretados/secretados já revelaram a presença de uma ampla variedade de proteínas com potencial imunomodulador (AGUIAR-SANTOS; MEDEIROS; BONFIM; ROCHA *et al.*, 2013; CRUZ; MARCILLA; KELLY; VANDENPLAS *et al.*, 2021).

Deste modo, acredita-se que a análise integral do proteoma de *T. trichiura* adultosmacho e fêmea, juntamente com a avaliação de proteínas candidatas selecionadas, produzidasde forma recombinante e testadas em PBMCs de indivíduos alérgicos, possa gerar dados e produtos imunoterapêuticos em um futuro próximo. Produtos que sejam úteis no tratamento de doenças alérgicas, inflamatórias, autoimunes e na saúde pública de maneira mais abrangente.

4 OBJETIVOS

4.1 GERAL

Avaliar o proteoma do parasito adulto *T. trichiura* e o potencial imunomodulador de uma proteína selecionadas *in silico* e produzida de forma recombinante.

4.2 OBJETIVOS ESPECÍFICOS

- Caracterizar e descrever o proteoma do parasito, com análise de segregação por gênero, a fim de obter uma compreensão abrangente de suas propriedades estruturais e funcionais.
- Selecionar *in silico* uma molécula com perfil adequado para terapias inflamatórias e, em seguida, produzir essa molécula candidata de forma recombinante.
- Avaliar a imunorreatividade da molécula recombinante produzida.
- Dosar, quantificar e avaliar o perfil de citocinas em PBMCs após estimulação utilizando a molécula selecionada.

5. PROTEOMA DO VERME ADULTO DE *Trichuris trichiura* : UMA FONTE POTENCIAL DE MOLÉCULAS IMUNOMODULADORAS

O estudo proteômico envolve a análise sistemática do conjunto de proteínas de um determinado organismo, célula ou sistema biológico, visando determinar suas características, quantidades e funções. A diferenciação proteômica e a identificação das moléculas envolvidas nos processos parasitários de diversos helmintos permitem uma compreensão mais profunda da relação desses parasitos com seus hospedeiros. Isso resulta em informações valiosas e perspectivas significativas no campo da pesquisa de biomoléculas e em suas diversas aplicações. A análise proteômica apresenta vantagens em comparação à análise genômica e do transcriptoma uma vez que, após o processamento, as proteínas podem sofrer modificações pós-traducionais que precisam ser consideradas ao analisar sequências genéticas (MONTANO; CUELLAR; SOTILLO, 2021; MONTANO; LOUKAS; SOTILLO, 2021).

Os produtos derivados de helmintos representam uma fonte promissora de biomarcadores e biomoléculas com propriedades imunomoduladoras. Essas substâncias são produzidas por meio de vias bioquímicas exclusivas, moldadas ao longo de milênios de coevolução entre os parasitos e os seres humanos (WANGCHUK; YESHI; LOUKAS, 2023). Em trabalho de nosso grupo foi possível observar a produção de moléculas imunomoduladoras em monócitos do sangue periférico humano (PBMCs) estimulados com diferentes frações proteicas do verme adulto de *T. trichiura*. Além disso, foi possível identificar, por meio de LC-MS/MS, quais proteínas estavam envolvidas nesse processo (SANTOS; GALLO; SILVA; FIGUEIREDO *et al.*, 2013). Trabalhos como este abrem novas perspectivas para a compreensão da interação entre parasitos e o sistema imunológicohumano, com implicações importantes na pesquisa biomédica e no desenvolvimento de terapias inovadoras baseadas em moléculas naturais, potencialmente transformadoras para o tratamento de doenças alérgicas, inflamatórias e autoimunes.

Neste capítulo será apresentado um estudo onde LC-MS/MS foi utilizada em conjunto com ferramentas de bioinformática e ontologia genética para avaliar e descrever o proteoma dos vermes adultos de *T. trichiura* macho e fêmea. Adicionalmente, uma proteína ainda não caracterizada localizada nesse proteoma foi selecionada, produzida de forma recombinante e seu perfil imunomodulador foi avaliado em PBMCs.

5.1 ARTIGO: THE PROTEOME OF HUMAN ADULT WHIPWORM *Trichuris trichiura*: A SOURCE OF POTENTIAL IMMUNOMODULATORY MOLECULES.

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The proteome of human adult whipworm *Trichuris trichiura*: a source of potential immunomodulatory molecules

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Abstract

Soil-transmitted helminths (STHs), including *Trichuris trichiura*, are a major global health challenge, particularly impacting children and causing significant morbidity. However, the ability of *T. trichiura* to influence the host's immune responses presents an exciting opportunity to discover biomolecules with therapeutic potential for inflammatory, allergic, and autoimmune disorders. This study conducted a comprehensive proteomic analysis of adultmale and female *T. trichiura* worms using liquid chromatography-tandem mass spectrometry (LC-MS/MS), identifying 810 parasite proteins. Of these, 117 were exclusive to females, 277to males, and 356 shared between both genders.

Gene ontology analysis revealed that male and female parasites exhibited similar cellular component profiles, primarily involving intracellular structures. Nevertheless, female-exclusive proteins displayed more diverse cellular components. Molecular function analysis

emphasized the prevalence of hydrolytic and catalytic activities, suggesting potential enzymatic strategies employed by *T. trichiura* for nutrition and immune response modulation.Notably, proteins with immunomodulatory potential were identified in both genders, including a Kunitz protease inhibitor and glutamate dehydrogenase, showing promise for therapeutic applications. To evaluate the immunomodulatory potential, one of the identified proteins (rc4299) was tested using peripheral blood mononuclear cells (PBMCs) from allergic individuals. The recombinantly produced rc4299 protein exhibited immunomodulatory properties, notably increasing IL-10 secretion, indicating its potential application in treating autoimmune and allergic diseases. This study not only elucidates the intricacies of the *T. trichiura* proteome but also reveals promising therapeutic targets, highlighting the intricate interplay between the parasite and the host's immune system.

Keywords

Proteome, *Trichuris trichiura*, Immunomodulatory proteins

1 Introduction

Infection by soil-transmitted helminth (STHs) is a serious public health problem, especially in areas where environmental factors and social conditions favor the spread and persistence of infection at a high level. *Trichuris trichiura* is one of the most important STHs affecting humans, being responsible for approximately 465 million infections worldwide (1).

Trichuriasis in humans is generally asymptomatic, but it can manifest with abdominal pain, diarrhea, and, in more severe cases, Trichuris dysentery syndrome (TDS), characterizedby iron-deficiency anemia, chronic mucoid diarrhea, bleeding, and rectal prolapse. Children are the most affected by the disease, and even mild cases can result in physical and intellectual damage (2, 3).

was determined that the presence of the parasite in the intestine offers protection against immune diseases such asulcerative colitis (4) and multiple sclerosis (5). Viable egg intake of *T. trichiura* for the treatment of ulcerative colitis can reduce symptomatic colitis by promoting Type_2 immunity-dependent goblet cell hyperplasia and mucus production (6), and also led to the production of Interleukin 10 (IL-10) and Transforming Growth Factor Beta (TGF-β), especially when infection is chronic (7). *T. trichiura* is capable of modulatingthe host's immune

response through a mixed mucosal intestinal T cell response (type 1 T helper (Th1), Th2, Th17, and regulatory T (Treg) cells), with the production of cytokines and chemokines related to this profile, and a predominantly Th2 profile of circulating T helper cells (8, 9).

This ability to regulate the immune system has been shown to be promising, especially for inflammatory, allergic, and autoimmune diseases, where modulation of immunity sufficiently depresses immunological reactivity, thus reducing the symptomatology of these clinical disorders (10). However, these findings have significant relevance and pointout that *T. trichiura* infection is useful in relieving the symptoms of inflammatory diseases, the treatment with parasite eggs and the ideal conditions for any clinical benefit is still uncertain and it is unclear whether it is possible that the infection may exacerbate the existing pathological conditions (11).

Proteomic approaches have been employed to investigate the protein profile of *T. trichura* and its relationship with the immuneresponse of the infected individual. Recently, the proteome of the adult somatic extract (12) and the proteome of egg extracts (13) identified a variety of parasite-derived molecules that play a crucial role in regulating the host's immune response, revealing a diversity of moleculeswith immunomodulatory potential.

Although the proteome of secreted and excreted products, as well as the egg extract of *Trichuris* spp., have already been studied and revealed immunomodulatory molecules, these analyses represent only specific moments of the infection. The proteome of the parasite at other stages of its life cycle within the host has not yet been evaluated (13). The adult wormis in direct contact with host cells, and its proteome may reveal a wide array of molecules interacting with intestinal microbiota and host cells, leading to immunomodulation. In this study, we employed, for the first time, a proteomic approach to characterize the proteome of male and female *Trichuris trichiura* adult worms. Moreover, an uncharacterized molecule from the parasite, located within this proteome, was synthesized and assessed for its potential as an immunomodulator. This comprehensive investigation of the complete proteome of *Trichuris trichiura* has unveiled a diverse array of molecules. These findings hold the promise of contributing to thedevelopment of novel pharmaceuticals with utility in the treatment of allergic and inflammatory diseases, alongside advancements in diagnostic and vaccine development.

2 Materials and methods

2.1 Parasites collection from infected patients

Adult worms were collected in stool samples from infected children of the province of Esmeraldas - Ecuador, with Kato-Katz method positive for *T. trichiura* eggs.The children were treated with pyrantel pamoate and bisacodyl (14, 15). In addition, the worms (approximately 5 g - wet weight) were washed in 0.15 M phosphate-buffered saline (pH 7.4), and stored at -80℃ until use. Ethical approval for obtaining stool samples from the children was provided by the Ethics Committee of the San Francisco University of Quito, Ecuador. Informed written consent was provided by parents or guardians (12, 16, 17).

2.2 Total protein extraction from male and female worm

The adult worms was categorized between males and females (6). Then the worms were washed in 0.15 M phosphate buffered saline (PBS) pH 7.4 and three females and three males worms were separated into falcon-type tubes, per sex. The parasite lysis was performed in two steps. Firstly, a thermal shockand mechanical maceration were performed in five cycles of freezing-thawing in liquid nitrogen and at room temperature in the presence of a cocktail of protease inhibitors (Sigma- Aldrich, St. Louis MO, USA). Secondly, worm lysates were disrupted by a sonicator with 70 Hz amplitude pulses in PBS containing PMSF (Sigma Aldrich, St Louis MO, USA). The protein lysates were centrifuged at 12000 × g for 7 minutes at 4 ^{0}C to collect supernatants. Protein concentration was estimated by Bradford Protein Assay (Sigma-Aldrich, St. Louis MO, USA) and analyzed by 12% Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-Page).

2.3 Liquid chromatography and mass spectrometry (LC-MS/MS)

Samples containing the total extracts of the *T. trichiura* worms were digested with the ProteoExtract® All-in-One Trypsin Digestion Kit (Merk Millipore Darmstadt He, DE) according to the manufacturer's instructions. Peptides generated by proteolysis were separated and desalted by reverse-phase nano-HPLC Dionex Ultimate 3000 (Thermo FisherScientific, Waltham Ma, USA). The eluate resulting from the HPLC was directed directly via nanoelectrospray to a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham Ma, USA). The capillary voltage was 2 kV. For peptide identification, a top 12 data-dependent analysis method was used. The instrument was tuned to maximum sensitivity. The normalized fragmentation energy was 27%.

2.4 Data analysis and protein identification

Survey and fragment spectra were analyzed with Proteome Discover Version 1.4 (Thermo Fisher Scientific, Waltham Ma, USA) with SequestHT as the search engine and PEAKS Studio 8 (Bioinformatics Solutions, Waterloo, ON, Canada). Only peptides with high confidence scores (XCorr ≥ 2.3 for SequestHT, −10lgP ≥ 35 for PEAKS) were considered. The searches were compared with the *T. trichiura* annotated transcriptome database. All analyses were carried out in duplicates. OmixBox (70. https://www.biobam.com/omicsbox) was used to analyze the identity and similarity of the proteins located in this proteome and forfunctional classifications.

Subsequently, for a better understanding of structures, physical-chemical characteristics, andpossible immunoreactivity of the uncharacterized protein Trichuris_c4299 identified in a previous work (12, 14). bioinformatics platforms were used. The BLASTX (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to identify homologous sequences, the ProtParam (https://web.expasy.org/protparam/) to evaluate the physicochemical parameters, PSORT (http://www.psort.org/) to determine cellular localization, SignalP (http://services.healthtech.dtu.dk/services/SignalP-5.0/) and SOSUI (http://bp.nuap.nagoya-u.ac.jp/sosui/) to identify signal peptide and transmembrane helices and BCPREDS (http://ailab.ist.psu.edu/bcpred/predict.html) to predict the continuous B-cell epitopes.

2.5 Production of the recombinant protein rc4299

The hypothetical protein Trichuris_c4299 (14) shares homology with a immunomodulatory protein discovered in the excretory/secretory (E/S) product of *T. suis* (51). The coding sequence corresponding to the protein located in the proteome and selected *in silico* was synthesized commercially and cloned into the expression vector pET-28a (+) (GenScript, USA). The plasmid containing the Trichuris_c4299 nucleotide sequence was transformed by thermal shock in BL21(DE3) *Escherichia coli* strain. Protein expression wasperformed by cultivation in 1 L of Luria Bertani medium at pH 7.4 containing 50 µg/ml of kanamycin at 37 ºC, to which 0.5 mM isopropylb-D-1-thiogalactopyranoside (IPTG) was added (Thermo Scientific, Waltham, MA, USA) and the culture was shaken for 4 hours. Protein expression was confirmed in 12% SDS-PAGE, followed by Western blotting (WB), as described by Alves (18) and Salazar (19). After confirmation of expression of the recombinant protein, corresponding bacterial pellets were treated with solutions for native and denaturing conditions to determine solubility. Supernatants were analyzed by 12% SDS-Page. The Trichuris_rc4299 protein was purified by automated affinity chromatography on AKTA Pure25 from GE Healthcare (GE

Healthcare, Bio-Sciences AB, Björkgatan Uppsala, Sweden) witha HisTrap FF column (GE Healthcare, Bio-Sciences AB, Björkgatan Uppsala, Sweden) usingan elution buffer (10 mm Na_2HPO_4, 10 mM NaH_2PO_4, 8 M urea and 500 mM imidazole). Dialysis was performed on an AKTA Pure 25 GE Healthcare using a desalting column (GE Healthcare, Bio-Sciences AB, Björk-gatan Uppsala, Sweden) with 10 mM phosphate buffer. Dosage of recombinant protein was performed with the Quibit protein assay (Thermo Fisher Scientific, Waltham Ma, US), and the recombinant protein was stored at – 70 ºC until use.

2.6 Immunoreactivity evaluation by Dot Blot assay.

The immunoreactivity of protein Trichuris_rc4299 was evaluated by Dot-blot using serum of *T.trichiura*-positive patients. In brief, a nitrocellulose membrane Hybond-C Extra (GE Healthcare, Bio-Sciences AB, Björkgatan Uppsala, Sweden) was used for coating with 5 μg of recombinant protein rc4299, as well as 5 μg of *T. trichiura* worm extract (positive control).The spots were created using the Bio-Dot® Microfiltration System (BIO-RAD Life Science, Hercules, CA, USA). Blocking was performed using PBS with 5% skim milk for 1 hour; then, the membrane was washed 3 times with washing buffer containing PBS/ 0.05% Tween-20. The membrane was incubated with a pool of 10 sera of *T. trichiura*-positive patients at a 1/100dilution in PBS/ 0.05% Tween-20 for 4 hours. For detection of the reaction, the anti-human IgG antibody conjugated with peroxidase Zymax TM (Thermo Fisher Scientific, Waltham, MA, USA) was used diluted 1/500 in a blocking buffer. Development was carried out with 3'3' Diaminobenzidine (DAB) (1 mg/ mL PBS 1X pH 7.4) containing 10 μL of hydrogen peroxide. After the dot blot the image was captured by ImageQuant LAS 500 (GE Life Sciences, Piscataway, NJ, USA).

The sera from subjects not infected by *T. trichiura* were from healthy individuals who did not have *T. trichiura* eggs detected in feces. The sera from these negative controls and also from individuals with positive coproscopy for *T. trichiuris* were obtained from the sera bank of the Laboratory of Allergy and Acarology belonging to the Federal University of Bahia. Clearance no. 120.616.

2.7 Culture of peripheral blood mononuclear cells (PBMC) and cytokine analyses

A different set of patients was used for these assays. They were classified into allergic (n= 6) or non-allergic (n = 6) according to clinical history, the presence of specific IgE (sIgE)to Dermatophagoides. *pteronyssinus* in the ImmunoCAP® assay (Phadia Diagnostics AB,

Uppsala, Sweden), and positivity in skin prick test (SPT) for *D. pteronyssinus* extract. The study was approved by the Ethics Committee on Research of the Faculty of Medicine of the Federal University ofBahia, (CAAE 45376814.0.0000.5577).

For the cell culture, 20 mL of peripheral human blood from 12 individuals were dilutedin PBS with 2 mM EDTA. Then, the diluted blood was carefully layered on top of 15 mL of Ficoll-Paque PLUS (GE Life Sciences, Piscataway, NJ, USA). The tubes were centrifuged at880 g for 10 minutes at 4 °C in a swinging-bucket rotor. Immediately after centrifugation, thelayer formed in the interphase between Ficoll-Paque and cell suspension was gently collectedand transferred to another tube. Cells were washed with RPMI medium (Gibco, Pisley, UK) twice, by centrifugation at 1200 g for 30 minutes at 4 °C. Afterwards, cells were resuspendedin RPMI medium supplemented with 2% fetal bovine serum, 10 mM glutamine, and antibiotics (Sigma Chemical Co., St.Louis, Mo, USA). Finally, cell viability was assessed with 2% Trypan blue in Neubauer chamber. Cells were incubated in 96-well plates (2×10^5 cells/well) in a humidified atmosphere of 5% CO_2 at 37 °C in supplemented RPMI 1640 medium (GIBCO, Grand Island, NY, USA). Polymyxin B was used in almost all wells, apart from positive controls. This antibiotic inhibited the effects of LPS present in samples of the recombinant Cells were maintained in culture for 120 hours with supplemented medium aloneor stimulated with 25 µg/mL of rc4299; each of the conditions analyzed was carried out in 3 wells. Other antigens were used as cell culture controls. Cells were stimulated for 120 hours with 20 µg/mL of DpE or 10 µg/mL of pokeweed mitogen (PWM) (20, 21). Supernatants were used to quantify a total of 11 cytokines, which were detected simultaneously using a multiplex human cytokine/chemokine magnetic bead panel (MILLIPLEX MAP HumanCytokine/Chemokine Magnetic Bead Panel - Immunology Multiplex Assay, Millipore),following the manufacturer's instructions. Mean fluorescent intensities were determined on aMAGPIX® System and the concentration was calculated according to the standard curve andanalyzed using MILLIPLEX® Analyst 5.1 (Merck KGaA, Darmstadt, Germany).

3 Results and Discussion

3.1 Proteomic analysis of male and female *T. trichiura*.

The significant burden represented by STHs, particularly *T. trichiura*, on global public health has been well-established (22). Chronic infections, particularly in children, result in significant morbidity. However, the capacity of this parasite to modulate the host immune

response presents a promising avenue for the identification of valuable biomolecules in therapeutic interventions for inflammatory, allergic, and autoimmune disorders (23).

This is the first description of the total adult worm proteome of male and female *T. trichiura*. The protein lysates from male and female *T. trichiura* worms were separately analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). This process facilitated the identification of a total of 810 unique parasite proteins. Among this total, 117 proteins were exclusively identified in the female parasite, 277 were exclusively identified in the male parasite, and 356 were identified as beingshared between both sexes (Fig. 1).

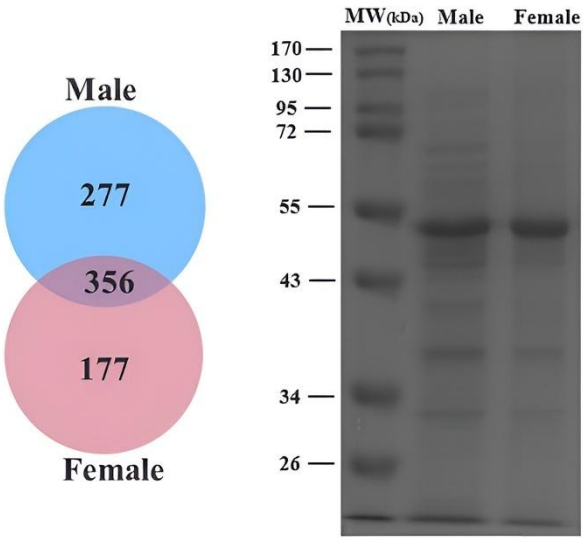

Fig. 1: Venn diagram of *T. trichiura* proteins identified by proteomics. Coomassie blue stained gel of protein extracts from male and female adult worms of *T. trichiura.* Lane (left to right): Mw, marker; Male, *T. trichiura* male adult worm; Female, *T. trichiura* female adult worm.

Significant difference was observed in the number of exclusively identified proteins between the male (277 proteins) and female (177proteins) parasites. This numerical disparity may be associated with A limitations inherent to theproteomics approach, particularly in the initial protein extraction phase or the trypsinization process required for LC-MS/MS analysis. These limitations could result in the underrepresentation of small proteins, low-abundance proteins, and poorly soluble proteins during detection. (24). However, other studies assessing the helminth proteome, *Schistosoma mekongi* (24, 25), *Schistosoma japonicum* (26), *Schistosoma mansoni* (27), *Angiostrongylus cantonensis* (28) and *Heligmosomoides polygyrus bakeri* (29), identified a higher number of proteins in male parasites compared to females, suggesting a potential biological distinction between the genders and their respective roles within the host.

3.2 Gene ontology (GO) *analysis of male and female T. trichiura proteomes*

The proteome was classified and categorized according to gene ontology using the OmixBox® software, thus allowing the evaluation of biological processes, molecular functions, and cellular components. This approach made it possible to conduct an individualized analysis of the shared proteome and scrutinize its specific characteristics in each gender. The main proteins identified with their molecular functions, biological processes,and cellular components are provided in Table 1 (both genders), Table 2 (male), and Table 3(female). Only the top 30 proteins presenting the highest coverages and confidence of identification are shown.

Table 01: The top 30 proteins identified in *Trichuris trichiura* worm adult Male and Female extract proteome by LC-MS/MS.

Accession number [a]	Description	Peptides	MW (kDa)	Signal Peptide	Gene Ontology (GO) Annotations[b]		
					Molecular function	Biological Process	Cellular component
A0A077Z6U9	Myosin tail family protein	208	225529		Myosin		Myosin filament
A0A077YYK1	Protein unc g protein unc f protein unc d prot ein unc b protein unc a	116	755131		Protein kinase activity	Protein phosphorylation	
A0A077YWU8	Spectrin alpha chain	110	281264		Calmodulin binding	Actin filament capping	Cytoskeleton
A0A077Z6U0	Intermediate filament protein ifa 1	109	70711				Intermediate filament
A0A077Z5Q5	Poly-cysteine and histidine tailed protein isofor m 2	108	50495				
A0A077Z2C7	Protein asteroid	104	30674	01-23			
A0A077ZIM1	Tropomyosin	102	87298				
A0A077YWX2	Gal-bind lectin domain containing protein	97	363427		Carbohydrate binding		
A0A077Z9D4	Laminin EGF and I-set and Laminin G 2 and Laminin B and ig and Ig 2 and Ldl recept a domain containing prote in	90	305279		Calcium ion binding		
A0A077Z5B7	DUF1136 and I-set and Ig 2 domain containing prot ein	90	400614				
A0A077Z8E1	Paramyosin	86	101488		Muscle protein		Myosin complex
A0A077YX57	Enolase	76	49513		Phosphopyruvate hydratase activity	Glycolytic process	Phosphopyruvate hydratase complex
A0A077ZJF5	Muscle cell intermediate filament protein OV71	75	64439				Intermediate filament
A0A077Z7M0	Phosphoenolpyruvate carboxykinase GTP	73	70975		Phosphoenolpyruvate carboxykinase (GTP) activity	Gluconeogenesis	
A0A077Z8E4	Heat shock protein 70	71	130299		Structural constituent of ribosome	Response to stress	Ribonucleoprotein complex
A0A077Z544	Uncharacterized protein	65	33553	01-23			

A0A077ZLF1	Protein disulfide-isomerase	63	55730		Protein disulfide isomerase activity		Endoplasmic reticulum lumen
A0A077Z5U2	Malic enzyme	62	62847		NAD binding		
A0A077Z2S6	Protein mig c protein mig b protein mig a	59	235047		Serine-type endopeptidase inhibitor activity	Extracellular matrix organization	Extracellular region
A0A077YWK8	Alpha-1 4 glucan phosphorylase	57	101447		Glycogen phosphorylase activity	Glycogen metabolic process	
A0A077YY36	Talin 1	55	289013		Structural constituent of cytoskeleton	Cell adhesion	Cytoskeleton
A0A077Z3F9	ELFV dehydrog and ELFV dehydrog N domain containi ng protein	54	115590		Glutamate dehydrogenase	Amino acid metabolic process	
A0A077YYL1	Uncharacterized protein	52	27669			-	-
A0A077Z8G8	78 kDa glucose regulated protein	47	72784	01-18	ATP binding	Response to stress	Endoplasmic reticulum lumen
A0A077Z424	Protein TNT-2 isoform a (Fragment)	47	39302			Regulation of muscle contraction	Troponin comple
A0A077Z1F6	Heat shock protein 90	47	82924		ATP-dependent protein folding chaperone		
A0A077YXK0	Endoplasmin	47	93862	01-30	ATP-dependent protein folding chaperone		
A0A077ZHV3	Glyceraldehyde-3-phosphate dehydrogenase	46	37536		Oxidoreductase	Glycolytic process	
A0A077YWE3	Succinate dehydrogenase [ubiquinone] flavoprotein subunit mitochondrial	45	70717		Flavin adenine dinucleotide binding	Electron transport chain	Mitochondrial inner membrane
A0A077ZFT7	Calponin family protein	45	47976				

a: Protein accession number in accordance with the UniProt database.
b: Gene Ontology (GO) Annotations in accordance with the software Omixbox database.

In the cellular component category, the majority of proteins shared between the male and female parasites belong, respectively, to the classes of intracellular anatomical structures (26.0%), organelles (24.0%), and cytoplasmic proteins (18.0%), (Fig. 2). In the integral proteome of both genders, the proteins profiles demonstrated a similarity with the cellular component profile of shared male and female proteins (supplementary Figure 1). The most abundant protein unique to the male whipworm proteome was the spectrin beta chain, a structural protein associated with the cytoskeleton (Table 2). This cellular structure constitutes approximately 9.0% of the total cellular component proteins the integral proteome in each worm gender of *T. trichiura* (supplementary Figure 1), evidencing the importance of structuralintegrity for the parasite survival.

Proteins such as actin, tropomyosin, paramyosin, muscle cell intermediate filament protein, and filamin C, which are intimately associated with the cytoskeleton and muscles, are essential for the motility of helminth parasites (31). They have been extensively identified in this study (Supplementary Tables 1, 2, and 3). Furthermore, proteins linked to the cytoskeleton

and muscles were also frequently found in *T. trichiura* egg extract proteome (13), in egg secretions of *S. mansoni* (32), somatic extract of adults of *Trichinella spiralis* (33) and *Trichinella britovi* (34).

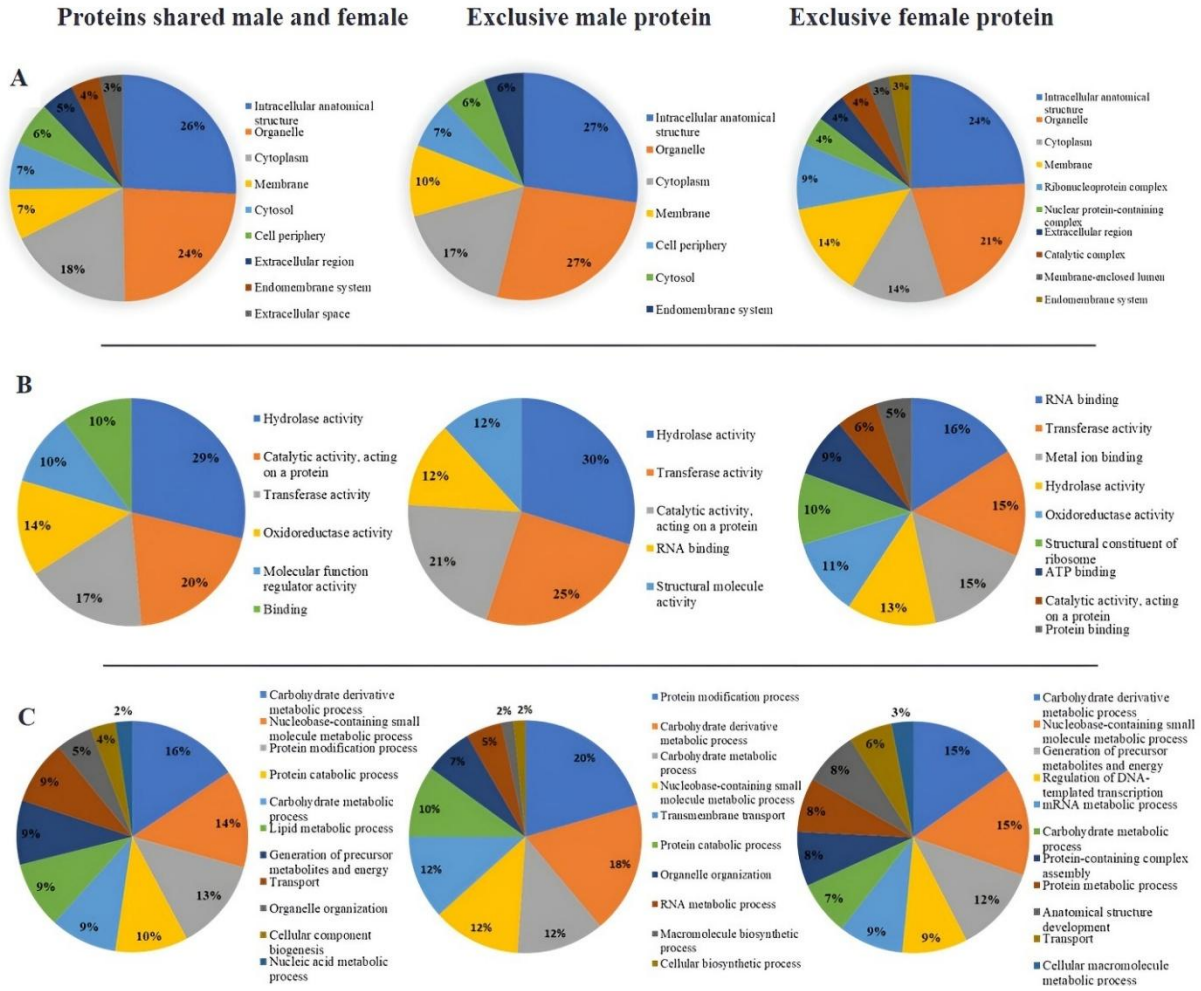

Fig. 2: Proteome classification of *Trichuris trichiura* shared and exclusive male and female adult worms.Proteomes were classified according to gene ontology including cellular component (**A**), molecular function (**B**)and biological process (**C**) categories.

Although the integral proteomes exhibit a similar cellular component profile, in the exclusive proteome of females, there is a greater diversity and distribution of cellular components compared to the exclusive proteome of males. The cellular components associated with the ribonucleoprotein complex, nuclear protein-containing complex, catalytic complex, and membrane-enclosed lumen were identified exclusively in the female parasite's proteome, demonstrating greater diversity in cellular components despite the female parasite having a minor proteome in absolute numbers (Figures 1 and 2).

The functional analysis of the integral proteome revealed the presence of a variety of

proteins with diverse molecular functions (Tables 1, 2, and 3). Among the male and female shared proteins, the primary identified proteins were with hydrolytic activity (29%), catalytic activity acting on a protein (20%), transferase activity (17%), and oxidoreductase activity (13%), respectively (Fig. 2). The predominance of proteins with hydrolytic and catalytic activity in the shared proteome points towards the potential enzymatic strategies employed by *T. trichiura* to aid in its nutrition, survival, and potentially modulate host immune responses. Through the higher-level of classification (level 2), it was possible to observe that the shared molecular function more present was the catalytic activity (70%) (supplementary Figure 2). The molecular function profile reached by the high rates of proteins with catalytic activity is consistent in both integral proteomes of male and female parasites (supplementary Figure 1), and the same function was observed in the unique proteome of male parasite (Figure 1). However, in the unique female proteome the higher-level classification (Level 2) revealed a predominance of binding activity (49%) to nucleic acid, ribonucleotide, and ion, surpassing catalytic activity (36%) (supplementary Figure 2).

Table 02: The top 30 proteins identified in *Trichuris trichiura* worm adult Male extract proteome by LC-MS/MS.

Accession number [a]	Description	Peptides	MW (kDa)	Signal Peptide	Gene Ontology (GO) Annotations[b]		
					Molecular function	Biological Process	Cellular component
A0A077Z6Z1	Spectrin beta chain	83	263326		Structural constituent of cytoskeleton	Actin filament capping	Membrane
A0A077Z7M3	Muscle M line assembly protein unc 89	83	754551		Guanyl-nucleotide exchange factor activity		
A0A077ZAD0	Filamin C	38	243568		Actin cytoskeleton organization	Actin filament binding	Intracellular non-membrane-bounded organelle
A0A077ZFD3	Methylmalonyl coenzyme A mutase mitochondrial	30	76674		Cobalamin binding		
A0A077Z5D8	T complex protein 1 subunit alpha	22	61528		Unfolded protein binding		
A0A077Z2V8	Phosphotransferase	21	52647		Glucose binding	Glycolytic process	
A0A077Z242	Cpn60 TCP1 domain containing protein	21	60486		Protein folding chaperone		Cellular Component
A0A077ZD87	T complex protein 1 subunit eta	20	153172		Transcription coregulator activity		Cytoplasm
A0A077ZGW0	T complex protein 1 subunit beta	20	58989		Unfolded protein binding		Chaperonin-containing T-complex
A0A077ZCT9	Calcium-transporting ATPase	19	102020		P-type calcium transporter activity		Sarcoplasmic reticulum membrane
A0A077ZEA4	Pyruvate kinase	18	62615		Pyruvate kinase activity		

A0A077Z3T2	Oxoglutarate:malate carrier protein	17	33551		Membrane		
A0A077YY59	Eukaryotic initiation factor 4A	17	49871		Translation initiation factor activity		P granule
A0A077ZI04	Guanine nucleotide binding protein subunit	17	36193		Translation regulator activity		Ribosome
A0A077Z8X2	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit	16	48787	01-17		Protein N-linked glycosylation via asparagine	Endoplasmic reticulum membrane
A0A077YYH9	Ubiquitin activating enzyme E1	16	122330		Ubiquitin-like modifier activating enzyme activity	Protein ubiquitination	
A0A077YYH2	Rab GDP dissociation inhibitor	15	50034		Rab GDP-dissociation inhibitor activity	Protein transport	Cytoplasm
A0A077YWA6	Oxidoreductase zinc binding dehydrogenase	15	39998		oxidoreductase activity		
A0A077ZCQ3	Apoptosis inducing factor 1 mitochondrial	14	63970		Oxidoreductase activity	Apoptotic process	Mitochondrion
A0A077Z4K9	Mannosyl oligosaccharide glucosidase	14	93798		Glycosidase	Protein N-linked glycosylation	Endoplasmic reticulum membrane
A0A077ZJA4	His Phos 1 domain containing protein	13	40507				
A0A077ZIY8	Lamin B1	12	63043				Intermediate filament
A0A077YYW4	UTP--glucose-1-phosphate uridylyltransferase	12	52652		Nucleotidyltransferase	Glycogen biosynthetic process	
A0A077ZAI4	Cathepsin D aspartic protease	12	44950	01-17	Aspartyl protease	Proteolysis	
A0A077ZK25	40S ribosomal protein S3a	11	29945		Structural constituent of ribosome	Translation	Cytoplasm
A0A077ZEK1	Hypoxia up regulated protein 1	11	100793		Protein folding chaperone	Response to stress	
A0A077Z5H0	Protein disulfide isomerase A4	11	67470	01-20	Isomerase		Endoplasmic reticulum lumen
A0A077Z4A2	Plasma alpha l fucosidase	11	54555	01-17	Alpha-L-fucosidase activity	Fucose metabolic process	
A0A077Z0M1	Uncharacterized protein	11	18556				
A0A077YX42	Sulfhydryl oxidase	10	211701	01-23	Oxidoreductase	Phenylalanyl-tRNA aminoacylation	Mitochondrial matrix

a: Protein accession number in accordance with the UniProt database.
b: Gene Ontology (GO) Annotations in accordance with the software Omixbox database.

It is worth noting that vitellogenin, a protein with lipid transporter activity, consideredone of the main components of *T. trichiura* eggs (13), was the protein that exhibited the highest peptide coverage in this study. This protein is one of the most abundant and is exclusively located in the female worm's proteome (Table 3). Considering that adult female *T. trichiura* can release thousands of eggs per day, it is not surprising to identify proteins involved in

reproductive processes as one of the most significant. This protein was among themost highly transcribed genes in the *T. trichiura* adult worm transcriptome (14), it was highlyexpressed in the transcriptome of adult female *Trichuris muris*, particularly localized in the posteriorportion of the worm's body (35), egg extract proteome *T. trichiura* (13), secretomes of adult *Ascaris suum* (36), and *Ancylostoma ceylanicum* (30).

In addition, the biological process analyzes of shared proteins from both gender proteomes demonstrated that the major of proteins are involved in the carbohydrate derivativemetabolic process (16%), nucleobase-containing small molecule metabolic process (14%), and protein modification process (13%), (Figure 2). The carbohydrate metabolism processesare highly conserved and impact nearly every aspect of helminth biology (37, 38). A metabolomic study in *A. suum*, *Ascaris lumbricoides*, *Necator americanus*, *Toxocara canis*, *S. mansoni*, *T. muris*, and other helminths demonstrated that both in adult worms and in infectious stages, these parasites primarily rely on carbohydrate and aminoacid metabolism to sustain their biological processes (39).

Table 03: The top 30 proteins identified in *Trichuris trichiura* worm adult Female extract proteome by LC-MS/MS.

Accession number[a]	Description	Peptides	MW (kDa)	Signal Peptide	Gene Ontology (GO) Annotations[b]		
					Molecular function	Biological Process	Cellular component
A0A077ZE83	Vitellogenin N and VWD and DUF1943 domain contain ing protein	231	198525	01-19	Lipid transporter activity		
A0A077ZFY4	TIL and CBM 14 domain containing protein	23	196047	01-31	Chitin binding		
A0A077Z814	Tyrosinase domain containing protein	23	52556	01-19	Oxidoreductase activity		
A0A077Z8B3	CBM 14 domain containing protein	14	78597	-	Chitin binding		Extracellular region
A0A077Z0R1	Uncharacterized protein	12	17867	01-19			
A0A077Z6Z8	Bifunctional purine biosynthesis protein	8	65983	-	Transferase	Purine biosynthesis	
A0A077Z3K5	TSP 1 and CBM 14 domain containing protein	8	93432	-	Chitin binding		Extracellular region
A0A077YXT2	Uncharacterized protein	6	69581	01-18			
A0A077Z8V8	Cadherin C and Laminin G 2 and Cadherin domain co ntaining protein	6	287046	-	Calcium ion binding	Morphogenesis of an epithelium	Plasma membrane
A0A077ZE65	Cpn10 domain containing protein	5	10387	-	ATP-dependent protein folding chaperone		
A0A077ZFK2	CBM 14 domain containing protein	5	192211	-	Chitin binding		Extracellular region
A0A077Z8Q1	HSP20 domain containing protein	5	12202	-			
A0A077Z453	Uncharacterized protein	4	172002	-			
A0A077YZY9	Ribosomal protein L15	4	29101	01-16			Ribosome
A0A077ZIB0	Uncharacterized protein (Fragment)	4	41240	-			
A0A077YVV8	StAR-related lipid transfer protein 5	4	28390	-	Lipid binding		

A0A077YUJ5	Kunitz protease inhibitor	4	27117	01-18	Serine-type endopeptidase inhibitor activity		
A0A077Z7B4	Chymotrypsin-elastase inhibitor ixodidin	4	10198	-	Serine protease inhibitor	Negative regulation of peptidase activity	Extracellular region
A0A077Z178	Uridine phosphorylase 1	3	68627	-	Uridine phosphorylase activity	Nucleotide catabolic	Cytoplasm
A0A077ZH59	SecE and NusG and KOW and Ribosomal L11 N and Rib osomal L11 domain containing protein	2	44573	-	Structural constituent of ribosome	Translation	Ribosome
A0A077Z7Y2	Histidine acid phosphatase family protein	2	47712	-			
A0A077ZBM2	Putative casein kinase substrate phosphoprotein p p28	2	22505	-	Kinase activity	Phosphorylation	
A0A077Z2P1	Uncharacterized protein	2	18802	01-16			
A0A077Z572	Charged multivesicular body protein 2a	2	24961	-		Vacuolar transport	
A0A077Z9X8	RNA polymerase II transcriptional coactivator	2	11679	-	Transcription coactivator activity	Positive regulation of transcription initiation by RNA polymerase II	Nucleus
A0A077Z055	Uncharacterized protein	2	13931	-			
A0A077Z108	Peptidase C1 domain containing protein	2	28233	-	Cysteine-type peptidase activity	Proteolysis	
A0A077ZDQ6	Thioredoxin and ATP-synt ab and Rho N and Rho RNA bind domain containing protein	2	59233	-	RNA binding	DNA-templated transcription termination	
A0A077ZCC7	PNP UDP 1 domain containing protein	2	25950	-	Glycosyltransferase	nucleoside catabolic process	
A0A077YZ91	DUF788 and Tsg domain containing protein	2	44568	01-25			Extracellular region

a: Protein accession number in accordance with the UniProt database.
b: Gene Ontology (GO) Annotations in accordance with the software Omixbox database.

The classification of biological process of shared male and female proteins at level 2 showed proteins involved in the cellular process (46%): heat shock protein 70, chaperonin protein heat shock protein 60 and tubulin beta chain; followed by metabolic process (42%): enolase, glucose-6-phosphate isomerase, fructose-bisphosphate aldolase and cystatin proteins; and localization process (12%): talin 1, vinculin and plastin 3 proteins (supplementary Figure3 and Table 1). The biological process profile of proteome exclusively of male is similar to the biological process profile of shared proteins between genders (Figure 2, supplementary Figure 3 and supplementary Table 2). Notwithstanding, the biological process in the female proteome is distinct of male showing proteins involved in cellular process (38%), followed by metabolic process (37%), biological regulation (9%), regulation of biological process (8%) and localization process (7%) (supplementary Figure 3).

3.3 Potentially immunomodulatory molecules found in the proteome of *T. Trichiura*

In two previous studies of our group, we have identified proteins with immunomodulatory potential (12, 14). We also evaluated whether the same proteins were found in the top 30 proteins identified in the present proteomic study. Interestingly, all identified proteins with immunoregulatory activity were found in both genders, except for protein A0A077YUJ5, a Kunitz protease inhibitor, which was only found in the top 30 identified proteins in female *T. trichiura* (Table 3). This protein stood out because it was not found in our previous omics analyses (12, 14).

Kunitz protease inhibitor proteins are well-known ubiquitous serine protease inhibitors that present promising therapeutic utility, that usually target essential proteases involved in inflammation, antigen processing, and presentation or hemostasis, blocking them in a substrate-like manner (40-42). The protein Sm-KI from *S. mansoni*, for instance, has inhibited neutrophil function by decreasing both its migration and elastase activity (43). In a study of our group, Sm-KI displayed immunomodulatory ability in vitro by reducing the production of IL-5 while up-regulating IL- 10 secreted by PBMC from allergic donors. In vivo, the protein was able to reduce allergic inflammation of mice by down-regulation of IL-5 and IL-4 in lung homogenates and of serum IgE. This protein also decreased the levels of eosinophil peroxidase in lung homogenates and in the bronchoalveolar lavage (18). It is possible to infer that the *T. trichiura* Kunitz protease inhibitor found in the present study will behave similarly to Sm-KI, given that other counterparts showed similar immunomodulatory activity (41).

The protein with accession number A0A077Z3F9 displays glutamate dehydrogenase activity. This protein was identified in one protein fraction with immunomodulatory activity in our previous omics studies, but it was not one of the most expressed transcripts (12, 14). The literature states that several helminth glutamate dehydrogenases can control inflammation (44). The enzyme modulates Th2 responses by targeting eicosanoid pathways, upregulating PGE2 and IL-10, and downregulating cysteinyl leukotrienes in macrophages (44). Helminth glutamate dehydrogenase may also induce naïve T cells to polarize into T cell regulatory cells (45).

Another protein, with ID A0A077Z8E4, is essential to discuss. This is a heat shock protein 70, which was found in two protein fractions with immunomodulatory activity in our proteomic study (12). Interestingly, heat shock protein 70 transcripts were amongst the most highly expressed in the T. trichiura transcriptome (14). The protein is part of a group of ‘autoantigens’ potentially triggering immunoregulatory pathways, which can inhibit inflammatory responses in sicknesses, such as rheumatoid arthritis, type 1 diabetes, and possibly atherosclerosis and

allergy (46, 47). This protein is generally part of helminth extracellular vesicles, which are main immunomodulators, promoting IL-10 release by macrophages and they suppress effector T-cell proliferation (48-50).

Another protein identified in the present proteomic analysis that are also part of helminth extracellular vesicles is laminin G 2 and cadherin and EGF CA domain containing protein (ID A0A077Z9D4). However, in our previous proteomic analysis, the protein was found in one protein fraction only (12).

Three additional proteins were found among the top 30 identified proteins that may possess immunomodulatory properties. However, no studies were found in the literature testing such molecules as immunomodulators. The proteins were: (a) ID A0A077YYK1, which displays protein kinase activity, found in both genders. It was identified in one fraction with immunomodulatory activity as well as a transcript in our transcriptomic analysis; (b) ID A0A077Z7M0, a phosphoenolpyruvate carboxykinase GTP, which was present in three fractions and the most expressed transcript; and (c) ID A0A077Z6U0, an intermediate filament protein IFA-1, which was found in one fraction among the five more expressed transcripts (12, 14). It is warranted, therefore, to test these proteins in a recombinant form. Firstly, test them in human cell cultures and later in experimental models to confirm if their presence in the protein fractions really had an influence in the immunomodulation previously observed, given that several proteins were part of the fraction (12).

3.4 Uncharacterized proteins

Although we have found a significant number of proteins involved in several processes, other uncharacterized proteins were found in this work which require more in-depth studies to determine their possible biological functions (supplementary Table 4). Among them, uncharacterized proteins with accession numbers in UNIPROT database A0A077ZGB3 possess domains of possible trypsin-like activity similar to peptidase S1 of Trichuris suis A0A077ZGB3-1 was identified in this work.

This protein had previously been identified by mass spectrometry in the excretory/secretory extract from L1 *T. suis* larvae and exhibited immunomodulatory activity in vitro and in a murine model of allergy (51). Our discovery of the previously uncharacterized protein, Trichuris_c4299, in the proteome of *T. trichiura*, specifically its presence in both male and female worms, is essential to ascertain its existence and functionality, as its transcript was annotated as a hypothetical protein (14).

3.5 Production and immunoreactivity evaluation of the recombinant protein rc4299

The uncharacterized protein Trichuris_c4299 was recombinantly produced. The production and purification of the rc4299 protein was confirmed by SDS-PAGE and Western Blot techniques, presenting bands of ca. 30 kDa (Figure 3). The Dot-Blot results showed that sera from individuals infected with *T. trichiura* were reactive to the rc4999 protein, and also to the *T. trichiura* somatic extract (positive control) (Figure 3).

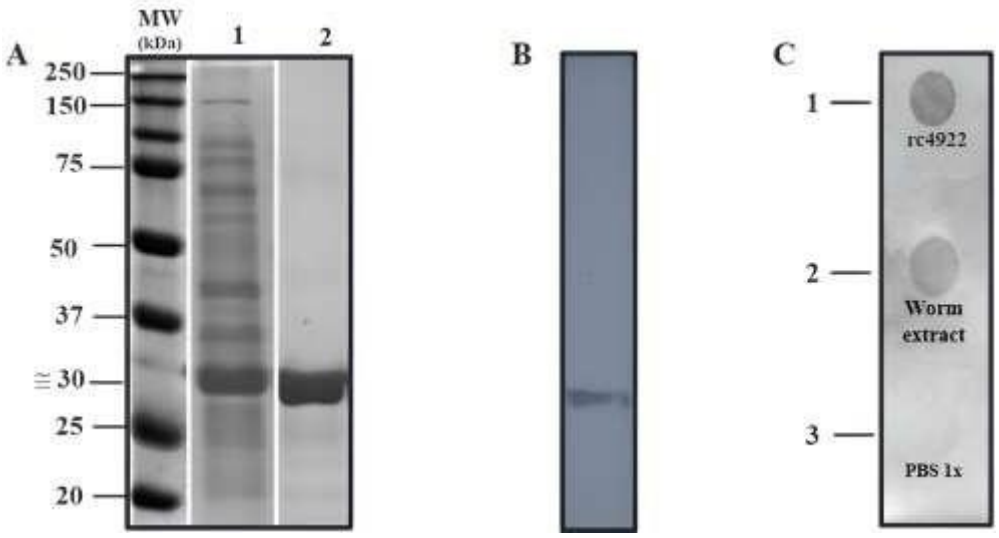

Fig. 3: (**A**) Coomassie blue stained gel of production and purification rc4922 of *T. trichiura*. **(B)** Western blotting using anti-6xHIS antibodies and anti-human IgG secondary antibody conjugated with peroxidase. (**C**) Dot-Blot using rc4922 protein (1), protein extracts from male and female adult worms of *T. trichiura* (**2**), negative control PBS 1x (**3**) and patient serum pool of *T. trichiura* infected.

Figures 4 and 5 show the baseline values (medium) as well as the values of cytokines and chemokines after *in vitro* stimulation of human cells with rc4299 protein. All cells secreted significantly greater amounts of all cytokines when a mitogen was used as a stimuli (data not shown), indicating the validity of the cell culture. Figure 4 reveals that rc4299 protein did not significantly induce the secretion of IL-4, IL-5, IL-13 and eotaxin. Considering the role of all these cytokines and chemokines in the immunopathology of allergic diseases (52-54), this lack of stimulation was an interesting result. As expected, it was observed that PBMCs from allergic patients secreted more IL-4 and IL-13, in a significant manner, than PBMCs from healthy donors (Figure 4). It is well known how IL-4 induces IgE production, given its role inpromoting the class-switch mechanism in plasmocytes (55). Additionally, IL-13 also participates in class-switch mechanism, but its main role is related to the hyperplasia of gobletcells and mucus production (56). Therefore, it was expected high basal amounts of this cytokines in allergic

donors.

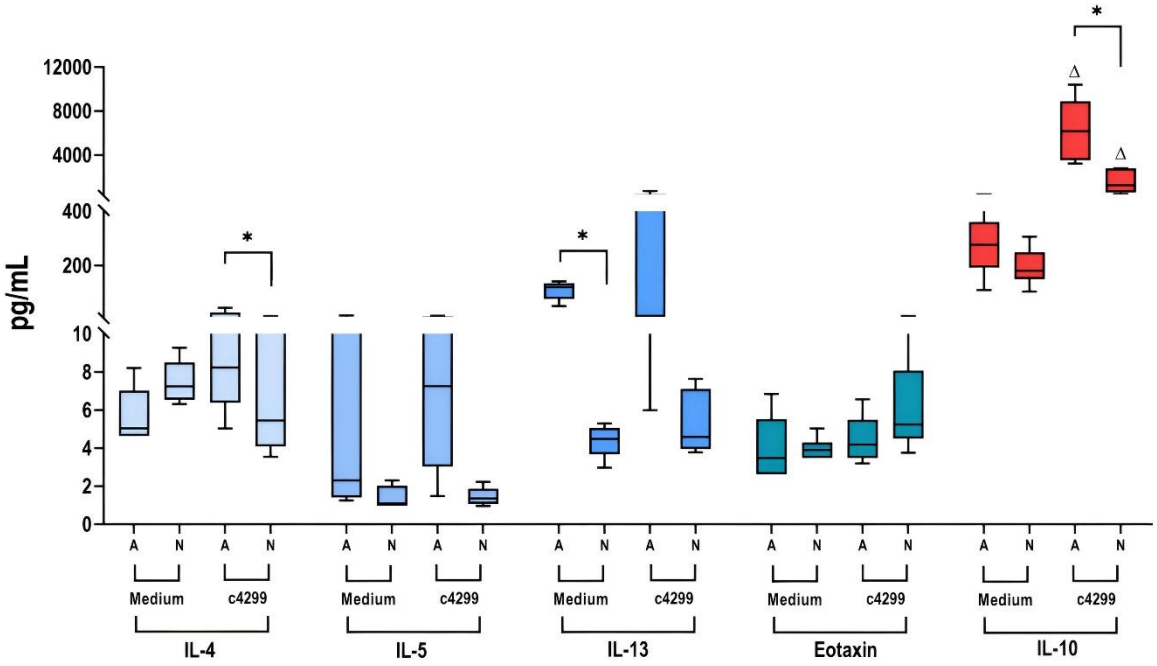

Fig. 4: Type 2 cytokines, eotaxin and IL-10 secretion in cultures of peripheral blood mononuclear cells from healthy and allergic individuals (N = 6, each) stimulated with c4299 from *T. trichiura*.The Milliplex Map Human Cytokine kit was used to measure the cytokines displayed at the X axis. The pairedt-test or Wilcoxon signed test was used to verify these statistical differences between unstimulated cells (Medium) and c4299-stimulated cells from the allergic group (A) and non-allergic group (N). The * symbol was used to show the significant differences. After this analysis, for comparisons between groups with the same stimulus, the unpaired t test or Mann-Whitney test was used. The Δ symbol was used to show the significant differences.

Another appealing *in vitro* feature of rc4299 protein was its ability to induce high levels of IL-10 compared to Medium, either by PBMC from allergic or from non-allergic donors (Figure 4). IL-10 is mainly considered a regulatory cytokine, acting as a potent regulator of inflammatory responses and playing a critical role in controlling allergic airway inflammation. In addition, the cytokine is able to induce the proliferation of both B and T regulatory cells, which, in turn, can regulate excessive immune responses (57, 58). On the other hand, IL-10 was more secreted by PBMC from allergic donors than PBMC from healthyindividuals. Although this result may be initially unexpected, it has been previously shown that not all IL-10 acts as a regulatory cytokine (59, 60). Nevertheless, our group has found similar results using recombinant hypoallergenic molecules as stimuli and this higher secretion upon recombinant protein stimulation may be a result of an already excessively reactive immune response. IL-10 higher secretion may be a way to in turn reduce the production of inflammatory cytokines (20, 21).

Among the main findings shown in Figure 5, we can firstly mention the high secretion

of IFN-α2 from PBMC of allergic donors upon stimulation with rc4299 protein. Although these types of cytokines may present a dubious behavior in autoimmunity, sometimes enhancing and other times preventing inflammation (61), in allergic diseases IFN-α's role seems to be clearer. In such illnesses, together with IFN-β, they block several fundamental Th2 mechanisms: activation of transcription factor GATA3; granulocyte activation; IL-4- mediated IgE production; production of IL-13 and IL-5 (62, 63). In addition, IFN-α may havean influence in upregulating IL-10 (64). A balance of Th1-biased and/or regulatory immuneresponses is indeed a feature expected in candidate molecule for allergen immunotherapy, analternative treatment for allergic diseases (20, 21).

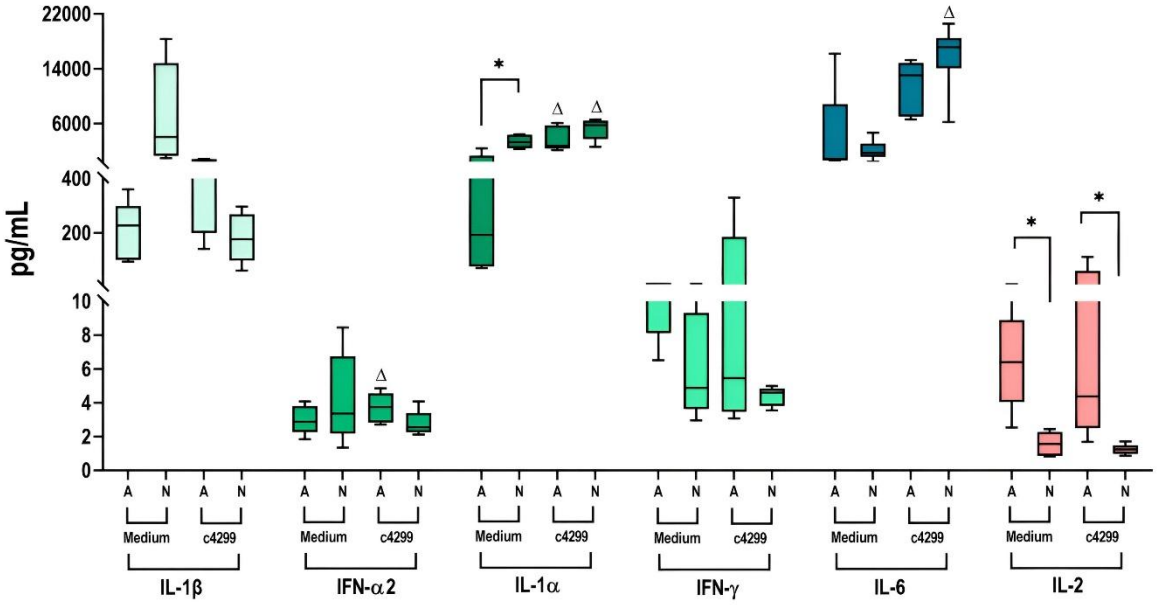

Fig. 5: Type 1 and innate cytokines secretion in cultures of peripheral blood mononuclear cellsfrom healthy and allergic individuals (N = 6, each) stimulated with c4299 from *T. trichiura*. The Milliplex MapHuman Cytokine kit was used to measure the cytokines displayed at the X axis. The paired t-test or Wilcoxon signed test was used to verify these statistical differences between unstimulated cells (Medium) and c4299- stimulated cells from the allergic group (A) and non-allergic group (N). The * symbol was used to show the significant differences. After this analysis, for comparisons between groups with the same stimulus, the unpairedt test or Mann-Whitney test was used. The Δ symbol was used to show the significant differences.

In this aspect, rc4299 protein appears to be an appealing therapeutic, given the high levels of IL-2, especially in allergic donors (Figure 5). Although there was no significant increase in comparison with the medium, the higher production in allergic donors upon rc4299 protein is an exciting result because the increase in IL-2 is an outcome that confirms the efficacy of therapeutics in allergen immunotheraphy. This cytokine influences the production of IL-10 and might induce shifts in T helper responses (65, 66). However, the increase observed for IL-1α and IL-6 is not as appealing, considering the role of such cytokines in allergen sensitization

(67-69).

4 Conclusion

Considering all the data herein, it can be concluded that proteomics is a reliable tool to evaluate differences in protein expression between *T. trichiura* genders. We confirm that while males showed a higher number of proteins, females displayed a more diversified repertoire of proteins, indicating the complexity needed for egg development. In addition, webelieve our proteomic analysis confirmed not only the presence of immunomodulatory proteins previously found, but also identified new possible targets for future studies, such as rc4299 protein, Kunitz protease inhibitor and Glutamate dehydrogenase. Among these new targets, we produced a recombinant version of protein rc4299 protein that clearly displayed appealing immunomodulatory features for its potential use in future therapeutic purposes, especially for the treatment of autoimmune and allergic diseases.

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Author contributions

All authors made substantial contributions to all of the following: CSP and FF made the conception and design of the study, LFS, ESS, PSS, PB, SPO, AMSF, RCS, VSA made or acquisition of data, or analysis and interpretation of data, CSP, LGCP, NMAN, LFS drafting the article and revising it critically for important intellectual content. All authors read and approval the final version to be submitted.

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Supplementary Material

The proteome of human adult whipworm *Trichuris trichiura*: a source of potentialimmunomodulatory molecules.

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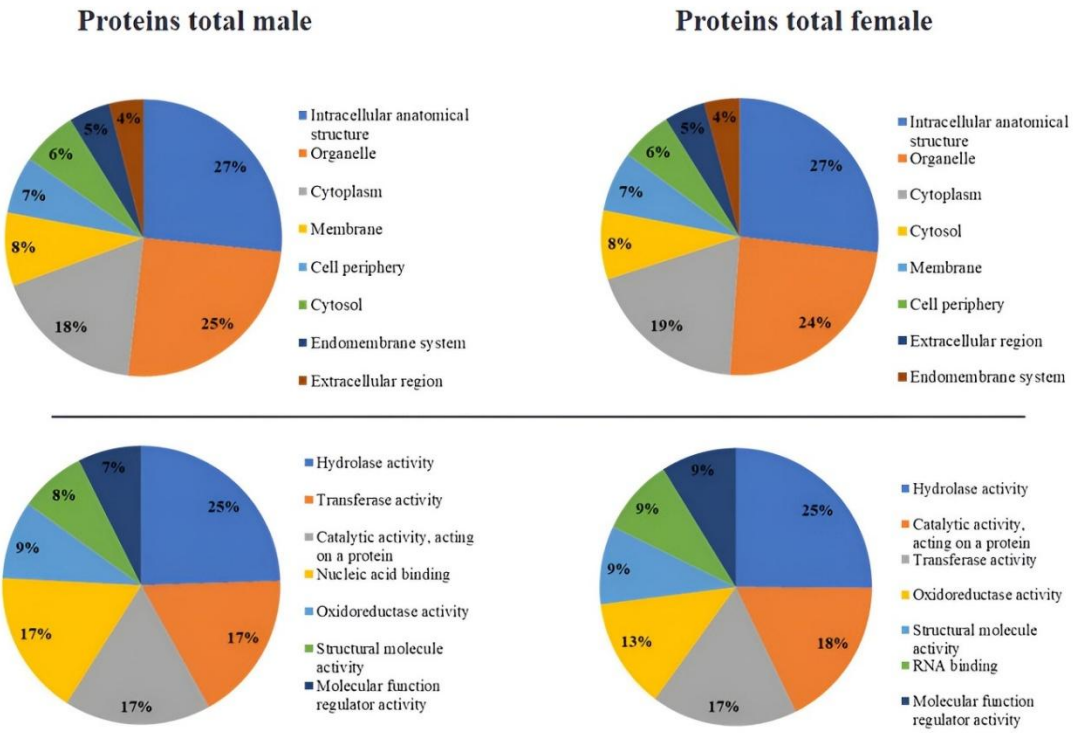
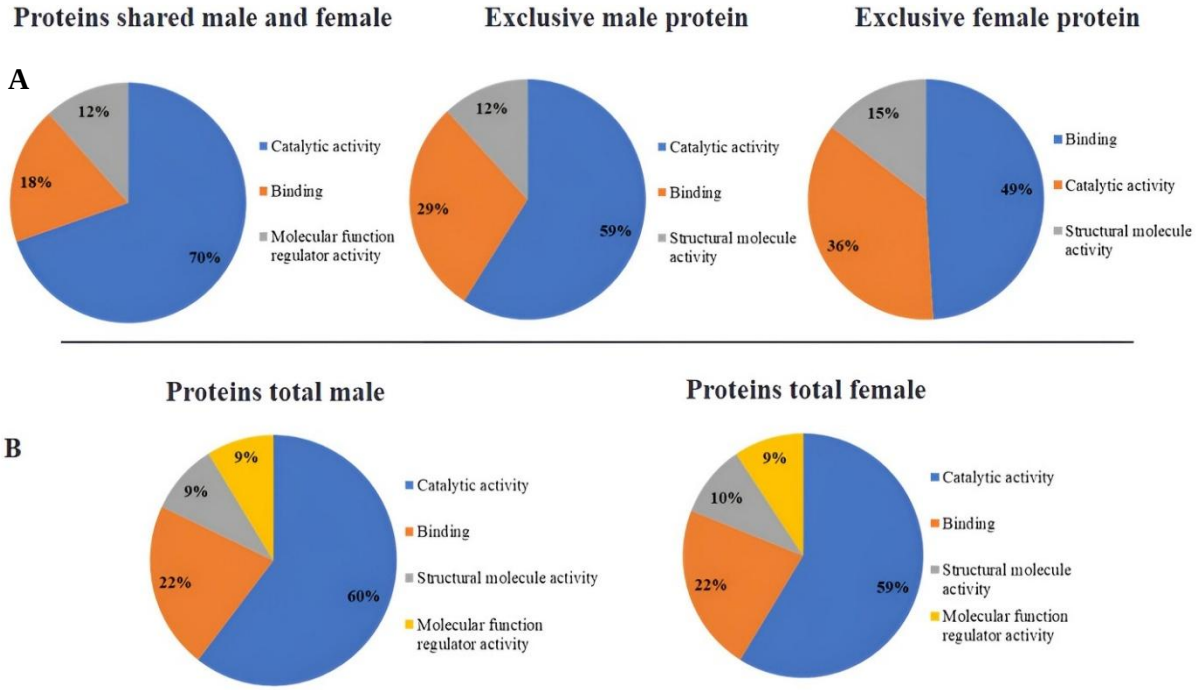
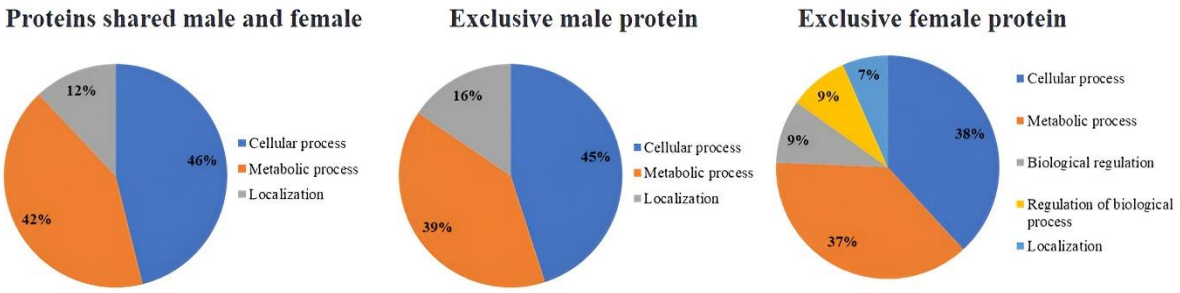


Supplementary Fig 1: Integral Proteome classification of *T trichiura* male and female adult worms. Proteomes were classified according to gene ontology including cellular component (**A**), molecular function (**B**) categories.



Supplementary Fig. 2: Proteome higher-level classification (Level 2) of *T. trichiura* accordingto gene ontology demonstrating molecular function in proteome shared an proteome exclusive male and femaleadult worms (**A**), as well as in integral proteome in male and female worms.



Supplementary Fig. 3: Proteome higher-level classification (Level 2) of *T. trichiura* accordingto gene ontology demonstrating biological process in proteome shared and proteome exclusive male and femaleadult worms.

Table supplementary 1: Proteins identified in Trichuris trichiura Male and Female extract proteome by LC-MS/MS using Proteome Discoverer Version 1.4 (Thermo Fisher Scientific) with SequestHT as the search engine and PEAKS Studio 8 (Bioinformatics Solutions, Waterloo, ON, Canada). and OmixBox Ontology database.

Description	Accession number[a]	Peptides	MW (kDa)	Signal Peptide	Molecular function[b]	Biological Process[b]	Cellular component[b]
Myosin tail family protein	**A0A077Z6U9**	208	226	-	Myosin	-	myosin filament
Protein unc g protein unc f protein unc d prot ein unc b protein unc a	**A0A077YYK1**	116	755	-	protein kinase activity	protein phosphorylation	-
Spectrin alpha chain	**A0A077YWU8**	110	281	-	calmodulin binding	actin filament capping	cytoskeleton
Intermediate filament protein ifa 1	**A0A077Z6U0**	109	71	-	-	-	intermediate filament
Poly-cysteine and histidine tailed protein isofor m 2	**A0A077Z5Q5**	108	50	-	-	-	-
Protein asteroid	**A0A077Z2C7**	104	31	01-23	-	-	-
Tropomyosin	**A0A077ZIM1**	102	87	-	-	-	-
Gal-bind lectin domain containing protein	**A0A077YWX2**	97	363	-	carbohydrate binding	-	-
Laminin EGF and I-set and Laminin G 2 and Laminin B and ig and Ig 2 and Ldl recept a domain containing prote in	**A0A077Z9D4**	90	305	-	calcium ion binding	-	-
DUF1136 and I-set and Ig 2 domain containing prot ein	**A0A077Z5B7**	90	401	-	-	-	-
Paramyosin	**A0A077Z8E1**	86	101	-	Muscle protein	-	myosin complex
Enolase	**A0A077YX57**	76	50	-	phosphopyruvate hydratase activity	glycolytic process	phosphopyruvate hydratase complex
Muscle cell intermediate filament protein OV71	**A0A077ZJF5**	75	64	-	-	-	intermediate filament

Phosphoenolpyruvate carboxykinase GTP	**A0A077Z7M0**	73	71	-	phosphoenolpyruvate carboxykinase (GTP) activity	gluconeogenesis	-
Heat shock protein 70	**A0A077Z8E4**	71	130	-	structural constituent of ribosome	response to stress	ribonucleoprotein complex
Uncharacterized protein	**A0A077Z544**	65	34	01-23	-	-	-
Protein disulfide-isomerase	**A0A077ZLF1**	63	56	-	protein disulfide isomerase activity	-	endoplasmic reticulum lumen
Malic enzyme	**A0A077Z5U2**	62	63	-	NAD binding	-	-
Protein mig c protein mig b protein mig a	**A0A077Z2S6**	59	235	-	serine-type endopeptidase inhibitor activity	extracellular matrix organization	extracellular region
Alpha-1 4 glucan phosphorylase	**A0A077YWK8**	57	101	-	glycogen phosphorylase activity	glycogen metabolic process	-
Talin 1	**A0A077YY36**	55	289	-	structural constituent of cytoskeleton	cell adhesion	Cytoskeleton
ELFV dehydrog and ELFV dehydrog N domain containi ng protein	**A0A077Z3F9**	54	116	-	glutamate dehydrogenase	amino acid metabolic process	-
Uncharacterized protein	**A0A077YYL1**	52	28	-	-	-	-
78 kDa glucose regulated protein	**A0A077Z8G8**	47	73	01-18	ATP binding	response to stress	endoplasmic reticulum lumen
Protein TNT-2 isoform a (Fragment)	**A0A077Z424**	47	39	-	-	regulation of muscle contraction	troponin comple
Heat shock protein 90	**A0A077Z1F6**	47	83	-	ATP-dependent protein folding chaperone	-	-
Endoplasmin	**A0A077YXK0**	47	94	01-30	ATP-dependent protein folding chaperone	-	-
Glyceraldehyde-3-phosphate dehydrogenase	**A0A077ZHV3**	46	38	-	Oxidoreductase	glycolytic process	-

Succinate dehydrogenase [ubiquinone] flavoprotein subunit mitochondrial	**A0A077YWE3**	45	71	-	flavin adenine dinucleotide binding	electron transport chain	mitochondrial inner membrane
Calponin family protein	**A0A077ZFT7**	45	48	-	-	-	-
Phosphoglycerate kinase	**A0A077Z3K7**	44	45	-	phosphoglycerate kinase activity	glycolytic process	-
Uncharacterized protein	**A0A077Z906**	43	39	01-17	-	-	-
Propionyl-CoA carboxylase alpha chain mitochondr ial	**A0A077ZBT2**	43	132	-	ligase activity	-	mitochondrial matrix
Calreticulin	**A0A077ZC09**	43	47	01-17	calcium ion binding	protein folding	endoplasmic reticulum lumen
Ank 2 and ZU5 and Ank domain containing protein	**A0A077Z1R9**	43	179	-	-	-	-
Independent phosphoglycerate mutase	**A0A077ZDB4**	43	58	-	phosphoglycerate mutase activity	glycolytic process	Cytoplasm
Dihydrolipoyl dehydrogenase	**A0A077Z970**	42	50	-	dihydrolipoyl dehydrogenase activity	-	-
Bravo FIGEY and I-set and fn3 and Ig 3 domain con taining protein	**A0A077Z439**	42	143	-	-	-	Membrane
Chaperonin protein heat shock protein60	**A0A077ZIE8**	41	63	-	ATP-dependent protein folding chaperone	protein refolding	-
Protein disulfide-isomerase	**A0A077ZJZ3**	39	55	01-18	protein disulfide isomerase activity	-	endoplasmic reticulum lumen
Malate dehydrogenase	**A0A077YZW9**	38	36	-	L-malate dehydrogenase activity	malate metabolic process	-
Glucose-6-phosphate isomerase	**A0A077ZIX8**	38	63	-	glucose-6-phosphate isomerase activity	glycolytic process	-
Fructose-bisphosphate aldolase	**A0A077Z6Y9**	38	41	-	fructose-bisphosphate aldolase activity	glycolytic process	-
Aldo ket red domain containing protein	**A0A077ZDK2**	37	269	-	oxidoreductase activity	-	-

Elongation factor 2	**A0A077Z0M0**	37	95	-	GTPase activity	-	-
Fumarate hydratase mitochondrial	**A0A077YZK2**	37	50	-	fumarate hydratase activity	fumarate metabolic process	tricarboxylic acid cycle enzyme complex
A macroglobulin complement component family	**A0A077Z549**	36	144	-	endopeptidase inhibitor activity	-	extracellular space
Pyruvate dehydrogenase E1 component subunit alpha	**A0A077YYR5**	34	42	-	pyruvate dehydrogenase (acetyl-transferring) activity	acetyl-CoA biosynthetic process from pyruvate	intracellular membrane-bounded organelle
Epididymal secretory protein E1	**A0A077Z0I4**	34	46	01-23	sterol binding	intracellular cholesterol transport	extracellular region
Troponin I 2	**A0A077ZDB6**	34	31	-	-	-	troponin complex
Elongation factor 1-alpha	**A0A077YYL7**	33	51	-	GTPase activity	-	Cytoplasm
EF hand family protein	**A0A077Z8X9**	33	35	01-19	calcium ion binding	-	sarcoplasmic reticulum lumen
L 3 hydroxyacyl coenzyme a dehydrogenase short	**A0A077Z595**	33	35	-	oxidoreductase activity	fatty acid metabolic process	-
Putative transforming growth factor-beta-induced protein ig-h3	**A0A077ZAA9**	32	77	01-22	-	-	-
14-3-3 protein	**A0A077YXJ9**	32	33	-	-	-	-
OV 16 antigen	**A0A077ZPX5**	31	20	-	-	-	-
Uncharacterized protein	**A0A077YZI4**	31	99	-	-	-	-
GST C and GST N domain containing protein	**A0A077ZC56**	30	43	-	glutathione transferase activity	-	-
Galectin	**A0A077YZM7**	30	32	-	carbohydrate binding	-	-
Cystatin	**A0A077Z255**	30	27	01-17	cysteine-type endopeptidase inhibitor activity	proteolysis	-
Cell division cycle protein	**A0A077ZLV1**	29	90	-	ATP hydrolysis activity	cell division	-
Tubulin beta chain	**A0A077ZJ06**	29	52	-	GTPase activity	microtubule-based process	microtubule

Peptidyl-prolyl cis-trans isomerase	**A0A077YZV4**	28	19	-	peptidyl-prolyl cis-trans isomerase activity	protein peptidyl-prolyl isomerization	-
Actin-depolymerizing factor 2 isoform c	**A0A077Z0N1**	28	35	-	actin binding	actin filament depolymerization	actin cytoskeleton
Glyco hydro 31 and Gal mutarotas 2 domain contain ing protein	**A0A077YZY3**	28	110	01-18	carbohydrate binding	carbohydrate metabolic process	-
Calreticulin	**A0A077ZFW3**	27	51	-	unfolded protein binding	protein folding	endoplasmic reticulum lumen
Propionyl coenzyme A carboxylase beta chain	**A0A077Z4W0**	27	60	-	ligase activity	-	-
Succinate dehydrogenase [ubiquinone] iron-sulfur subunit mitochondrial	**A0A077ZJC7**	27	32	-	succinate dehydrogenase (ubiquinone) activity	tricarboxylic acid cycle	mitochondrial inner membrane
ECH domain containing protein	**A0A077Z1N9**	26	31	-	catalytic activity	-	-
General vesicular transport factor p115	**A0A077Z8D1**	26	164	-	-	intracellular protein transport	Golgi membrane
Calsequestrin	**A0A077ZEY0**	25	49	01-21	calcium ion binding	-	sarcoplasmic reticulum lumen
ATP synthase subunit beta	**A0A077Z3I0**	25	55	01-20	proton-transporting ATP synthase activity	-	proton-transporting ATP synthase complex, catalytic core F(1)
Vinculin	**A0A077YX53**	25	107	-	structural molecule activity	cell adhesion	actin cytoskeleton
3 ketoacyl coenzyme A thiolase mitochondrial	**A0A077ZJA3**	25	43	-	acyltransferase activity	-	-
Calponin like protein	**A0A077YZI3**	25	17	-	-	-	-
Alpha N acetylgalactosaminidase	**A0A077Z3S2**	24	47	01-21	hydrolase activity	carbohydrate metabolic process	-

Small heat shock protein	**A0A077Z7R3**	24	18	-	-	-	-
Peptidyl-prolyl cis-trans isomerase	**A0A077Z7S3**	24	23	01-20	peptidyl-prolyl cis-trans isomerase activity	protein peptidyl-prolyl isomerization	-
Beta-hexosaminidase	**A0A077Z072**	24	63	01-20	N-acetyl-beta-D-galactosaminidase activity	carbohydrate metabolic process	-
Carbonyl reductase (Nadph) 1	**A0A077ZE80**	24	31	-	oxidoreductase activity	-	-
Major sperm protein	**A0A077ZIQ5**	23	19	-	-	-	cytoskeleton
PDZ and LIM domain containing protein	**A0A077YXN5**	23	79	-	metal ion binding	-	-
Acetyltransferase component of pyruvate dehydrogenase complex	**A0A077Z0S4**	23	44	-	dihydrolipoyllysine-residue acetyltransferase activity	acetyl-CoA biosynthetic process from pyruvate	mitochondrion
Superoxide dismutase [Cu-Zn]	**A0A077Z345**	23	15	-	superoxide dismutase activity	-	-
Aminopeptidase	**A0A077Z146**	23	111	-	metallopeptidase activity	proteolysis	-
Phosphoglucomutase	**A0A077YY22**	23	62	-	phosphoglucomutase activity	carbohydrate metabolic process	-
Type I inositol 1 4 5 trisphosphate	**A0A077Z559**	22	44	-	inositol-polyphosphate 5-phosphatase activity	-	-
Uncharacterized protein	**A0A077Z373**	22	34	01-20	-	-	-
4 hydroxybutyrate coenzyme A transferase	**A0A077ZC52**	22	51	-	acetyl-CoA hydrolase activity	acetate metabolic process	-
Alkali myosin light chain long isoform	**A0A077Z9G4**	22	17	-	calcium ion binding	-	-
Actin	**A0A077ZE37**	22	42	-	-	-	-
Pyruvate dehydrogenase component subunit beta mit ochondrial pyruvate dehydrogenase complex component pyruv ate dehydrogenase beta subunit	**A0A077ZCG5**	22	49	-	pyruvate dehydrogenase (acetyl-transferring) activity	acetyl-CoA biosynthetic process from pyruvate	mitochondrion

ADP ATP carrier protein heart:skeletal muscle	**A0A077ZJS2**	22	36	-	ATP:ADP antiporter activity	mitochondrial ATP transmembrane transport	mitochondrial inner membrane
Triosephosphate isomerase	**A0A077ZC84**	22	27	-	triose-phosphate isomerase activity	glycolytic process	-
Retinal dehydrogenase 2	**A0A077YZA6**	22	58	-	oxidoreductase activity	-	-
Nucleoside diphosphate kinase	**A0A077YZ73**	22	17	-	nucleoside diphosphate kinase activity	nucleoside diphosphate phosphorylation	-
Mediator of RNA polymerase II transcription subun it 22	**A0A077Z2H0**	21	15	01-19	-	-	cell surface
Ubiquitin associated and SH3	**A0A077ZFQ2**	21	37	-	-	-	-
Uncharacterized protein	**A0A077YW28**	20	36	01-22	-	-	-
Peroxiredoxin-2	**A0A077ZNK7**	20	22	-	peroxiredoxin activity	-	-
Trifunctional enzyme subunit alpha	**A0A077YXD7**	20	80	-	oxidoreductase activity	fatty acid beta-oxidation	-
NAD(P) transhydrogenase mitochondrial	**A0A077Z4N4**	20	114	-	NAD(P)+ transhydrogenase activity	proton transmembrane transport	membrane
Heat shock 70 kDa protein 4	**A0A077ZGB5**	20	81	-	ATP-dependent protein folding chaperone	response to stress	-
Low density lipoprotein receptor repeat	**A0A077Z260**	20	172	01-18	calcium ion binding	cell-matrix adhesion	membrane
Peptidylprolyl isomerase	**A0A077Z3W7**	20	49	-	peptidyl-prolyl cis-trans isomerase activity	-	-
Actin interacting protein 1	**A0A077Z717**	19	66	-	-	-	-
Fatty acid binding protein	**A0A077ZB79**	19	14	-	lipid binding	-	-
Methylmalonyl coenzyme A epimerase mitochondrial	**A0A077ZGS8**	19	16	-	-	-	-
60S ribosomal protein L18a	**A0A077Z189**	19	32	-	structural constituent of ribosome	translation	ribosome
Trans 2 enoyl coenzyme A reductase	**A0A077Z149**	19	41	-	oxidoreductase activity	-	-

Peptidase C1 domain containing protein	**A0A077ZGN1**	19	35	-	cysteine-type peptidase activity	proteolysis	-
Protein disulfide isomerase A6	**A0A077YWN0**	18	47	-	protein disulfide isomerase activity	-	endoplasmic reticulum
Ornithine aminotransferase	**A0A077Z4V5**	18	47	-	ornithine(lysine) transaminase activity	L-proline biosynthetic process	-
Moesin:ezrin:radixin 1	**A0A077ZIT0**	18	56	-	actin binding	-	cytoplasm
PDZ domain containing protein	**A0A077YWS0**	18	36	-	-	-	-
Adenylate kinase isoenzyme 1	**A0A077ZC00**	18	22	-	ATP binding	phosphorylation	-
Zf-C3HC4 and Lactamase B and zf-CCCH domain conta ining protein	**A0A077Z6W1**	18	69	-	metal ion binding	methylglyoxal catabolic process to D-lactate via S-lactoyl-glutathione	-
Heat shock 70 kDa protein 9 (Mortalin)	**A0A077ZM82**	18	48	-	ATP-dependent protein folding chaperone	response to stress	-
Insulin degrading enzyme	**A0A077ZCU5**	18	114	-	metalloendopeptidase activity	proteolysis	-
Putative heat shock protein	**A0A077ZCH5**	17	22	-	-	-	-
Transcription factor BTF3 4	**A0A077Z286**	17	75	-	-	-	-
Cystathionine beta synthase	**A0A077Z4J8**	17	57	-	lyase activity	cysteine biosynthetic process from serine	plasma membrane
Major sperm protein	**A0A077Z897**	17	25	-	-	-	cytoskeleton
Cupin 8 domain containing protein	**A0A077Z066**	17	60	01-16	-	-	-
DUF290 domain containing protein	**A0A077Z8H2**	17	18	01-19	-	-	-
Uncharacterized protein	**A0A077Z8F0**	16	31	-	-	-	-
HSP70 domain containing protein	**A0A077ZM07**	16	25	-	ATP binding	-	-
Cpn10 domain containing protein	**A0A077Z6Q7**	16	13	-	ATP binding	-	-
L-lactate dehydrogenase	**A0A077ZFV3**	16	37	-	L-lactate dehydrogenase activity	carboxylic acid metabolic process	cytoplasm

Copper:zinc superoxide dismutase (Fragment)	**A0A077Z0Y6**	15	17	-	superoxide dismutase activity	-	-
Troponin C	**A0A077Z6A8**	15	18	-	calcium ion binding	-	-
Collagen alpha (Iv) chain	**A0A077Z512**	14	172	-	extracellular matrix structural constituent	anatomical structure development	membrane
Uncharacterized protein	**A0A077Z6A6**	14	107	01-19	-	-	membrane
Protein CLP 1 d	**A0A077YVW3**	14	65	-	calcium-dependent cysteine-type endopeptidase activity	proteolysis	-
Trefoil and Glyco hydro 31 domain containing prot ein	**A0A077Z243**	14	104	01-19	carbohydrate binding	carbohydrate metabolic process	membrane
CathepsinC exc and Peptidase C1 domain containing protein	**A0A077Z0X8**	14	94	-	cysteine-type peptidase activity	proteolysis	-
Superoxide dismutase	**A0A077Z4X9**	14	22	-	metal ion binding	-	-
Plastin 3	**A0A077YW46**	14	68	-	actin filament binding	actin filament bundle assembly	-
T-complex protein 1 subunit delta	**A0A077YVX7**	14	60	-	unfolded protein binding	cytoplasm	-
Synthase subunit alpha, mitochondrial	**A0A077ZLW3**	13	25	-	proton-transporting ATP synthase activity	proton-transporting ATP synthase complex	-
Proliferating cell nuclear antigen	**A0A077Z0Q5**	13	32	-	DNA binding	DNA replication	nucleus
GDP L fucose synthase	**A0A077YXI3**	13	36	-	GDP-L-fucose synthase activity	'de novo' GDP-L-fucose biosynthetic process	-
NEDD8 activating enzyme E1 regulatory subunit	**A0A077ZIC3**	13	77	-	ubiquitin-like modifier activating enzyme activity	-	-
ATP synthase subunit alpha	**A0A077ZF69**	13	36	-	proton-transporting ATP synthase activity	-	proton-transporting ATP synthase complex
60S acidic ribosomal protein P0	**A0A077YWM4**	13	35	-	-	ribosome biogenesis	ribosome

60 kDa SS A:Ro ribonucleoprotein	**A0A077Z1S1**	12	72	-	RNA binding	-	cytoplasm
Uncharacterized protein	**A0A077Z482**	12	187	01-18	calcium ion binding	-	membrane
Myoglobin	**A0A077ZHY0**	12	16	-	oxygen carrier activity	-	-
Armet domain containing protein	**A0A077YZ13**	12	16	-	-	-	extracellular region
Adenosylhomocysteinase	**A0A077ZF21**	12	48	-	adenosylhomocysteinase activity	one-carbon metabolic process	-
DB domain containing protein	**A0A077YZI5**	12	15	-	-	-	-
V type proton atpase catalytic subunit a	**A0A077ZBL6**	12	61	-	proton-transporting ATP synthase activity	proton-transporting V-type ATPase	-
TsJ5	**A0A077YWH9**	12	81	-	RNA binding	-	-
Macrophage migration inhibitory factor	**A0A077Z686**	12	20	-	-	-	-
CAP and WH1 and PBD and WH2 domain containing pro tein	**A0A077ZDH4**	12	118	01-26	actin binding	actin filament organization	nucleus
Peptidylprolyl isomerase	**A0A077ZAC5**	12	12	-	peptidyl-prolyl cis-trans isomerase activity	-	-
Stress induced phosphoprotein	**A0A077Z724**	11	51	-	-	-	cytoplasm
Protein ETHE1 mitochondrial	**A0A077Z5Y3**	11	27	-	sulfur dioxygenase activity	glutathione metabolic process	-
Rhodanese domain containing protein	**A0A077Z538**	11	22	-	-	-	-
Major sperm protein	**A0A077Z9P9**	11	14	-	-	-	cytoskeleton
Ras protein Rab 1A	**A0A077YZ16**	11	23	-	GTPase activity	-	-
Methyltransf 31 domain containing protein	**A0A077Z5L8**	11	75	-	-	-	-
Ras protein rab 7a	**A0A077Z680**	11	24	-	GTPase activity	-	-
Apoptosis inducing factor 1 mitochondrial	**A0A077Z007**	11	71	-	oxidoreductase activity	apoptotic process	mitochondrion
Ferritin	**A0A077Z9L3**	11	21	-	ferroxidase activity	iron ion transport	-

Inositol-1-monophosphatase	**A0A077Z1R2**	11	30	-	inositol monophosphate 1-phosphatase activity	phosphatidylinositol phosphate biosynthetic process	-
Proteasome subunit alpha type	**A0A077Z826**	11	26	-	-	proteasome-mediated ubiquitin-dependent protein catabolic process	proteasome core complex
Major sperm protein	**A0A077ZFR0**	11	19	-	-	-	cytoskeleton
Major sperm protein	**A0A077ZFS2**	10	15	-	-	-	cytoskeleton
Trypsin and CUB domain containing protein	**A0A077Z376**	10	68	-	serine-type endopeptidase activity	proteolysis	-
Mannose-6-phosphate isomerase	**A0A077Z8R6**	10	48	-	mannose-6-phosphate isomerase activity	GDP-mannose biosynthetic process	-
Succinate--CoA ligase [ADP/GDP-forming] subunit alpha mitochondrial	**A0A077ZDU1**	10	36	-	nucleotide binding	tricarboxylic acid cycle	mitochondrion
Uncharacterized protein	**A0A077ZI12**	10	58	-	-	-	-
Adenylosuccinate synthetase	**A0A077YWW7**	10	50	-	adenylosuccinate synthase activity	'de novo' AMP biosynthetic process	cytoplasm
TIL domain containing protein	**A0A077Z4Z9**	10	31	01-22	serine-type endopeptidase inhibitor activity	negative regulation of peptidase activity	extracellular region
GTP-binding nuclear protein	**A0A077Z7J0**	10	24	-	GTPase activity	protein transport	nucleus
Iron dependent peroxidase	**A0A077Z6J2**	10	32	-	peroxidase activity	-	-
Profilin	**A0A077YX52**	10	15	-	actin binding	-	-
Phosphatidylethanolamine-binding protein	**A0A077YYR7**	10	19	-	-	-	-
Uncharacterized protein	**A0A077ZCJ0**	10	12	-	-	-	-
DUF290 domain containing protein	**A0A077Z5X5**	10	15	-	-	-	cell surface
HSP20 domain containing protein	**A0A077Z9T3**	**10**	44	-	-	-	-
ADH zinc N and ADH N domain containing protein	**A0A077Z4S4**	10	39	-	oxidoreductase activity	-	-

Protein-L-isoaspartate O-methyltransferase	**A0A077Z956**	10	27	01-20	protein-L-isoaspartate (D-aspartate) O-methyltransferase activity	-	-
Cathepsin Z	**A0A077ZDU9**	9	35	-	cysteine-type peptidase activity	proteolysis	-
Translationally controlled tumor protein	**A0A077Z8S9**	9	20	-	-	-	-
Peptidase M23 domain containing protein	**A0A077Z342**	9	37	-	metal ion binding	-	-
Protein SET	**A0A077Z633**	9	32	-	-	nucleosome assembly	nucleus
High mobility group protein 1,2	**A0A077Z3N6**	9	26	01-20	DNA binding	nucleus	-
GSHPx domain containing protein	**A0A077ZJY1**	9	42	-	glutathione peroxidase activity	response to oxidative stress	-
Glucosidase 2 subunit beta	**A0A077ZFW2**	9	46	-	-	-	membrane
Uncharacterized protein	**A0A077YZV0**	9	22	-	-	-	-
Eukaryotic translation initiation factor 5A	**A0A077ZAJ8**	9	18	-	ribosome binding	positive regulation of translational termination	-
Acyl coenzyme A thioesterase II	**A0A077Z3A8**	9	34	-	acyl-CoA hydrolase activity	acyl-CoA metabolic process	-
Ribosomal S19e domain containing protein	**A0A077Z148**	9	17	-	structural constituent of ribosome	translation	ribosome
Uncharacterized protein	**A0A077YVT6**	9	15	-	-	-	-
Papilin	**A0A077ZL56**	9	34	01-18	serine-type endopeptidase inhibitor activity	-	-
Hsc70 interacting protein	**A0A077ZK66**	9	29	-	protein dimerization activity	-	-
Charged multivesicular body protein 4b	**A0A077Z7D9**	9	25	-	-	-	vacuolar transport
Carbonic anhydrase	**A0A077YWY0**	9	30	-	carbonate dehydratase activity	-	-

Hypothetical FAD-dependent oxidoreductase YEL047C	**A0A077YVJ4**	9	57	-	oxidoreductase activity	-	-
Major sperm protein	**A0A077Z265**	9	22	-	-	-	cytoskeleton
Tyrosinase domain containing protein	**A0A077ZGT3**	9	85	-	oxidoreductase activity	-	-
Protein DJ 1	**A0A077ZA72**	9	20	-	-	-	-
Major sperm protein	**A0A077ZCB0**	9	14	-	-	-	cytoskeleton
Uncharacterized protein	**A0A077YYK9**	9	17	-	-	-	-
Major sperm protein	**A0A077YYW0**	9	15	-	-	-	cytoskeleton
GPR1:FUN34:yaaH family protein	**A0A077ZD64**	8	28	-	-	-	membrane
BAG family molecular chaperone regulator 2	**A0A077ZF57**	8	22	-	chaperone binding	-	-
Nucleobindin 1	**A0A077ZAK1**	8	62	-	calcium ion binding	cellular anatomical entity	-
Uncharacterized protein	**A0A077Z9S1**	8	96	-	-	-	-
Alcohol dehydrogenase NADP+ A	**A0A077Z8G1**	8	38	-	oxidoreductase activity	-	-
Aspartyl aminopeptidase (Fragment)	**A0A077ZIW8**	8	36	-	aminopeptidase activity	proteolysis	-
Uncharacterized protein	**A0A077Z0L5**	8	119	01-22	-	-	-
Uncharacterized protein	**A0A077ZAZ5**	8	58	01-24	-	-	-
Sb:cb283 protein	**A0A077Z4B7**	8	60	-	manganese ion binding	proteolysis	cytoplasm
40S ribosomal protein S14	**A0A077YZK6**	8	16	-	structural constituent of ribosome	translation	ribosome
UV excision repair protein RAD23-like protein B	**A0A077ZP79**	8	39	-	polyubiquitin modification-dependent protein binding	proteasome-mediated ubiquitin-dependent protein catabolic process	nucleoplasm
Protein rme isoform c protein rme isoform b pro tein rme isoform a	**A0A077ZBA9**	8	63	-	calcium ion binding	-	plasma membrane
Putative tumor protein d52 family	**A0A077Z173**	8	15	-	-	-	cellular anatomical entity
Ras protein Rab 2	**A0A077Z693**	8	24	-	GTPase activity	-	-

Thioredoxin mitochondrial	**A0A077ZIA0**	8	19	-	protein-disulfide reductase activity	-	-
Aspartate aminotransferase	**A0A077Z5G3**	8	48	-	L-aspartate:2-oxoglutarate aminotransferase activity	biosynthetic process	-
Chymotrypsin-elastase inhibitor ixodidin	**A0A077ZDY0**	8	8	-	serine-type endopeptidase inhibitor activity	negative regulation of peptidase activity	extracellular region
Serine protease inhibitor family protein	**A0A077Z583**	8	43	-	serine-type endopeptidase inhibitor activity	negative regulation of peptidase activity	membrane
Serpin domain containing protein	**A0A077ZDT9**	8	129	-	serine-type endopeptidase inhibitor activity	negative regulation of peptidase activity	extracellular space
Ras GTP binding protein Rho1	**A0A077Z7P8**	8	22	-	GTPase activity	small GTPase mediated signal transduction	-
40S ribosomal protein AS	**A0A077YZD4**	7	34	-	structural constituent of ribosome	translation	cytosolic small ribosomal subunit
VWA domain containing protein	**A0A077ZD14**	7	42	-	-	-	-
Probable nuclear transport factor nuclear transp ort factor (Ntf)	**A0A077YWY7**	7	15	-	-	mRNA transport	nucleus
Glucosylceramidase	**A0A077Z084**	7	61	01-18	glucosylceramidase activity	sphingolipid metabolic process	-
Y box-binding protein y box binding protein csd a protein	**A0A077Z7N0**	7	22	-	nucleic acid binding	-	-
ATP synthase-coupling factor 6 mitochondrial	**A0A077Z6H3**	7	24	-	proton transmembrane transporter activity	proton motive force-driven ATP synthesis	mitochondrial proton-transporting ATP synthase complex, coupling factor F(o)

Putative sorting nexin 13	**A0A077ZDZ6**	7	135	-	protein-ribulosamine 3-kinase activity	-	-
Major sperm protein	**A0A077Z7M4**	7	21	-	-	-	cytoskeleton
DUF1387 domain containing protein	**A0A077ZMZ6**	7	36	-	-	-	cytoplasm
Integrin beta	**A0A077YZG6**	7	49	-	-	integrin-mediated signaling pathway	membrane
Gut specific cysteine proteinase	**A0A077ZGS3**	7	40	01-19	cysteine-type peptidase activity	proteolysis	-
Proteasome subunit alpha type	**A0A077ZAK6**	7	29	-	-	ubiquitin-dependent protein catabolic process	nucleus
Alanine aminotransferase	**A0A077ZCV8**	7	59	-	transaminase activity	biosynthetic process	-
Adducin protein 1	**A0A077Z645**	7	61	-	-	-	-
40S ribosomal protein S7	**A0A077YW19**	7	20	-	structural constituent of ribosome	translation	ribonucleoprotein complex
CAP domain containing protein	**A0A077Z0T8**	7	34	-	-	-	-
Elongation factor 1 beta	**A0A077Z782**	7	24	-	translation elongation factor activity	-	eukaryotic translation elongation factor 1 complex
Ribosomal protein S28	**A0A077ZN86**	6	5	-	structural constituent of ribosome	translation	ribosome
Nascent polypeptide associated complex protein	**A0A077ZH89**	6	25	-	-	-	nascent polypeptide-associated complex
DUF290 domain containing protein	**A0A077ZLE4**	6	11	-	-	-	cell surface
Serpin B6	**A0A077ZG26**	6	65	-	serine-type endopeptidase inhibitor activity	serine-type endopeptidase inhibitor activity	extracellular space
Pyr redox dim and Pyr redox and Pyr redox 2 domai n containing protein	**A0A077YYR2**	6	70	-	thioredoxin-disulfide reductase	thioredoxin-disulfide reductase	-

Small heat shock protein	**A0A077Z6N1**	6	13	-	-	Stress response	-
40S ribosomal protein S18	**A0A077ZDK7**	6	18	-	RNA binding	translation	ribosome
Gut specific cysteine proteinase	**A0A077Z5F2**	6	40	-	cysteine-type peptidase activity	proteolysis	-
Oxidoreductase short chain	**A0A077ZAF4**	6	31	-	-	-	-
Uncharacterized protein	**A0A077YZ75**	6	14	-	-	-	-
Endostatin domain containing protein	**A0A077Z3G1**	6	53	-	-	-	-
Cystatin	**A0A077Z044**	6	15	01-17	cysteine-type endopeptidase inhibitor activity	-	-
Ras protein Rap 1b	**A0A077Z7Y5**	6	21	-	GTPase activity	Rap protein signal transduction	membrane
Proteasome subunit beta type 2	**A0A077YZS4**	6	24	-	proteolysis involved in protein catabolic process	-	proteasome core complex
Cystatin domain containing protein	**A0A077Z0W3**	6	11	-	cysteine-type endopeptidase inhibitor activity	-	cytoplasm
NUDIX domain containing protein	**A0A077ZG86**	6	16	-	bis(5'-nucleosyl)-tetraphosphatase activity	-	-
Proteasome subunit beta type	**A0A077YWJ6**	5	26	-	threonine-type endopeptidase activity	proteolysis involved in protein catabolic process	proteasome core complex
Ectonucleotide pyrophosphatase:phosphodiesterase	**A0A077ZCH4**	5	55	-	-	-	membrane
Endothelin converting enzyme 1	**A0A077ZLF3**	5	41	-	metalloendopeptidase activity	proteolysis	-
Uncharacterized protein	**A0A077ZKJ0**	5	65	-	-	-	-
NEDD8	**A0A077Z3R8**	5	12	-	-	-	-
Uncharacterized protein	**A0A077ZE99**	5	135	-	-	-	-
Eukaryotic aspartyl protease superfamily	**A0A077Z4E5**	5	34	01-23	aspartic-type endopeptidase activity	proteolysis	-

Integral ER membrane protein Scs2	**A0A077YZP2**	5	14	-	-	-	-
TAFII28 and Ribosomal L11 N and Ribosomal L11 dom ain containing protein	**A0A077ZKU5**	5	38	-	protein heterodimerization activity	translation	ribosome
ATPase inhibitor protein	**A0A077Z2I0**	5	14	-	ATPase inhibitor activity	negative regulation of ATP-dependent activity	mitochondrion
Transcription factor BTF3	**A0A077Z2S2**	5	18	01-21	-	-	-
40S ribosomal protein S12	**A0A077ZFS5**	5	15	-	structural constituent of ribosome	translation	ribosome
Xaa Pro aminopeptidase 3	**A0A077Z8W9**	5	72	-	metalloaminopeptidase activity	-	-
Acylphosphatase	**A0A077Z9X3**	5	12	-	acylphosphatase activity	-	-
TSP 1 domain containing protein	**A0A077ZB48**	5	103	01-21	-	-	-
Peptidase family m28 containing protein (Fragment)	**A0A077ZDI6**	5	36	-	glutaminyl-peptide cyclotransferase activity	-	-
p25 alpha family protein	**A0A077Z8D5**	5	22	-	tubulin binding	microtubule polymerization	-
Nudix hydrolase	**A0A077ZAF1**	4	31	-	ADP-ribose diphosphatase activity	-	-
Alpha L fucosidase	**A0A077Z127**	4	54	01-20	alpha-L-fucosidase activity	fucose metabolic process	-
Proteasome subunit alpha type	**A0A077ZDP8**	4	28	-	ubiquitin-dependent protein catabolic process	-	nucleus
Apolipoprotein A I binding protein	**A0A077ZAX5**	4	25	-	NADPHX epimerase activity	-	-
Complement component 1 Q subcomponent-binding pro tein mitochondrial	**A0A077ZC92**	4	22	-	-	-	mitochondrial matrix
Exo endo phos domain containing protein	**A0A077YZ15**	4	41	01-19	catalytic activity	-	-

Spermidine synthase	**A0A077Z3L8**	4	33	-	transferase activity	polyamine biosynthetic process	-
Ribosomal protein S17	**A0A077Z2N6**	4	19	-	structural constituent of ribosome	translation	ribosome
Death associated protein 1	**A0A077ZCY3**	4	11	-	-	-	-
N terminal Xaa Pro Lys N methyltransferase 1	**A0A077Z152**	4	69	-	protein methyltransferase activity	N-terminal protein amino acid methylation	-
Legume lectin family protein ergic family protei n	**A0A077Z597**	4	46	01-19	carbohydrate binding	-	membrane
I-set domain containing protein	**A0A077ZCI8**	4	12	-	-	-	-
Gut specific cysteine proteinase	**A0A077ZHU2**	4	41	01-17	cysteine-type peptidase activity	proteolysis	-
Proteasome endopeptidase complex	**A0A077Z7L4**	4	25	-	-	proteolysis involved in protein catabolic process	proteasome core complex
ATP synthase H+ transporting mitochondrial F1 c omplex delta subunit	**A0A077Z8I2**	4	18	-	proton-transporting ATP synthase activity, rotational mechanism	proton-transporting ATP synthase complex, catalytic core F(1)	-
Syntaxin 12	**A0A077Z5W5**	4	28	-	SNAP receptor activity	neurotransmitter transport	membrane
60S acidic ribosomal protein P2	**A0A077YX02**	4	9	-	structural constituent of ribosome	cytoplasmic translational elongation	cytosolic large ribosomal subunit
VWA domain containing protein	**A0A077Z2K2**	4	54	-	-	-	-
WAP domain containing protein SLPI-like	**A0A077YYJ8**	4	29	01-25	peptidase inhibitor activity	-	extracellular region
Galectin	**A0A077YZH6**	3	15	-	carbohydrate binding	-	-
Cytochrome b c1 complex subunit 6	**A0A077ZHB7**	3	8	-	-	mitochondrial electron transport, ubiquinol to cytochrome c	mitochondrial respiratory chain complex III

Spond N and Reeler and Kunitz BPTI and TSP 1 doma in containing protein	**A0A077Z3L6**	3	95	01-20	serine-type endopeptidase inhibitor activity	cell adhesion	extracellular region
Tissue factor pathway inhibitor 2 (Fragment)	**A0A077ZJB7**	3	84	-	serine-type endopeptidase inhibitor activity	-	-
Ribonuclease UK114	**A0A077Z8Z3**	3	14	-	-	-	-
Muscle LIM protein Mlp84B	**A0A077Z7W4**	3	19	-	metal ion binding	-	-
Ras protein let 60	**A0A077Z0B8**	3	27	-	GTPase activity	signal transduction	membrane
Myosin-2 essential light chain	**A0A077ZQT9**	3	21	-	calcium ion binding	-	membrane
Proteasome (Prosome macropain) subunit beta	**A0A077Z059**	3	22	-	threonine-type endopeptidase activity	proteolysis involved in protein catabolic process	proteasome core complex
40S ribosomal protein S21	**A0A077ZE43**	3	10	-	structural constituent of ribosome	translation	ribosome
Polyubiquitin	**A0A077ZCM7**	3	69	-	-	-	-
Thioredoxin	**A0A077Z1B4**	3	30	-	-	sensory perception	-
CAP domain containing protein	**A0A077Z1T9**	3	35	-	-	-	-
Gamma interferon inducible lysosomal thiol	**A0A077Z864**	3	29	01-18	oxidoreductase activity	-	-
Uncharacterized protein	**A0A077Z7J9**	3	132	01-25	-	-	-
Acyl carrier protein	**A0A077ZC49**	3	17	-	-	fatty acid biosynthetic process	-
Uncharacterized protein	**A0A077ZIV9**	3	132	-	-	-	-
UBX domain containing protein	**A0A077Z1Z1**	3	22	-	-	-	-
Uncharacterized protein	**A0A077ZGV8**	3	19	-	-	-	-
Cytochrome c oxidase polypeptide VIb	**A0A077Z344**	3	15	-	-	-	mitochondrion
NADH dehydrogenase (Ubiquinone) flavoprotein 2	**A0A077Z359**	3	21	-	oxidoreductase activity	-	-
Uncharacterized protein	**A0A077ZK49**	3	14	01-19	-	-	-

Small nuclear ribonucleoprotein Sm D3	**A0A077ZGZ0**	3	14	-	-	spliceosomal snRNP assembly	spliceosomal complex
Uncharacterized protein	**A0A077ZGB3**	2	32	01-20	-	-	-
UDP glucose pyrophosphatase	**A0A077YYA5**	2	17	-	-	-	-
Coronin	**A0A077ZFS4**	2	17	-	-	-	-
Abundant larval transcript 2 (Alt2) protein	**A0A077YY61**	2	20	01-29	-	-	-
Ubiquitin ribosomal protein putative ubiquitin f amily protein	**A0A077Z0U4**	2	15	-	structural constituent of ribosome	translation	ribosome
Protein CBG07648	**A0A077ZD81**	2	17	-	-	-	-
Uncharacterized protein	**A0A077Z4J9**	2	26	-	-	-	endosome membrane
Cystatin	**A0A077ZLD5**	2	13	01-18	cysteine-type endopeptidase inhibitor activity	-	-
Uncharacterized protein	**A0A077YZ92**	2	65	-	-	-	-
Cathepsin F	**A0A077Z0Q9**	2	70	01-17	cysteine-type endopeptidase inhibitor activity	proteolysis	-
Transcription state regulatory protein AbrB	**A0A077ZCJ5**	2	15	01-16	-	-	-

a: Protein accession number in accordance with the UniProt database.

b: Gene Ontology (GO) Annotations in accordance with the software Omixbox database.

Proteins that obtained at least 2 peptides identified by LC-MS/MS are described in the table. Preoteins with lower scores are not presented.

Table 2: Proteins identified in Trichuris trichiura Male extract proteome by LC-MS/MS using Proteome Discoverer Version 1.4 (Thermo Fisher Scientific) with SequestHT as the search engine and PEAKS Studio 8 (Bioinformatics Solutions, Waterloo, ON, Canada). and OmixBox Ontology database.

Description	Accession numbera	Peptides	MW (kDa)	Signal Peptide	Molecular functionb	Biological Processb	Cellular componentb
Spectrin beta chain	**A0A077Z6Z1**	83	263	-	structural constituent of cytoskeleton	actin filament capping	membrane
Muscle M line assembly protein unc 89	**A0A077Z7M3**	83	755	-	guanyl-nucleotide exchange factor activity	-	-
Filamin C	**A0A077ZAD0**	38	244	-	actin cytoskeleton organization	actin filament binding	intracellular non-membrane-bounded organelle
Methylmalonyl coenzyme A mutase mitochondrial	**A0A077ZFD3**	30	77	-	cobalamin binding	-	-
T complex protein 1 subunit alpha	**A0A077Z5D8**	22	62	-	unfolded protein binding	-	-
Phosphotransferase	**A0A077Z2V8**	21	53	-	glucose binding	glycolytic process	-
Cpn60 TCP1 domain containing protein	**A0A077Z242**	21	60	-	protein folding chaperone	-	Cellular Component
T complex protein 1 subunit eta	**A0A077ZD87**	20	153	-	transcription coregulator activity	-	cytoplasm
T complex protein 1 subunit beta	**A0A077ZGW0**	20	59	-	unfolded protein binding	-	chaperonin-containing T-complex
Calcium-transporting ATPase	**A0A077ZCT9**	19	102	-	P-type calcium transporter activity	-	sarcoplasmic reticulum membrane
Pyruvate kinase	**A0A077ZEA4**	18	63	-	pyruvate kinase activity	-	-
Oxoglutarate:malate carrier protein	**A0A077Z3T2**	17	34	-	membrane	-	-
Eukaryotic initiation factor 4A	**A0A077YY59**	17	50	-	translation initiation factor activity	-	P granule
Guanine nucleotide binding protein subunit	**A0A077ZI04**	17	36	-	translation regulator activity	-	ribosome
Dolichyl-diphosphooligosaccharide-protein glycosyltransferase 48 kDa subunit	**A0A077Z8X2**	16	49	01-17	-	protein N-linked glycosylation via asparagine	endoplasmic reticulum membrane
Ubiquitin activating enzyme E1	**A0A077YYH9**	16	122	-	ubiquitin-like modifier activating enzyme activity	protein ubiquitination	-

Rab GDP dissociation inhibitor	**A0A077YYH2**	15	50	-	Rab GDP-dissociation inhibitor activity	protein transport	cytoplasm
Oxidoreductase zinc binding dehydrogenase	**A0A077YWA6**	15	40	-	oxidoreductase activity	-	-
Apoptosis inducing factor 1 mitochondrial	**A0A077ZCQ3**	14	64	-	oxidoreductase activity	apoptotic process	mitochondrion
Mannosyl oligosaccharide glucosidase	**A0A077Z4K9**	14	94	-	Glycosidase	protein N-linked glycosylation	endoplasmic reticulum membrane
His Phos 1 domain containing protei	**A0A077ZJA4**	13	41	-	-	-	-
Lamin B1	**A0A077ZIY8**	12	63	-	-	-	intermediate filament
UTP--glucose-1-phosphate uridylyltransferase	**A0A077YYW4**	12	53	-	Nucleotidyltransferase	glycogen biosynthetic process	-
Cathepsin D aspartic protease	**A0A077ZAI4**	12	45	01-17	Aspartyl protease	proteolysis	-
40S ribosomal protein S3a	**A0A077ZK25**	11	30	-	structural constituent of ribosome	translation	Cytoplasm
Hypoxia up regulated protein 1	**A0A077ZEK1**	11	101	-	protein folding chaperone	response to stress	-
Protein disulfide isomerase A4	**A0A077Z5H0**	11	67	01-20	Isomerase	-	endoplasmic reticulum lumen
Plasma alpha l fucosidase	**A0A077Z4A2**	11	55	01-17	alpha-L-fucosidase activity	fucose metabolic process	-
Uncharacterized protein	**A0A077Z0M1**	11	19	-	-	-	-
Sulfhydryl oxidase	**A0A077YX42**	10	212	01-23	Oxidoreductase	phenylalanyl-tRNA aminoacylation	mitochondrial matrix
60S ribosomal protein L7a	**A0A077ZGS0**	10	32	-	RNA binding	ribosome biogenesis	cytosolic large ribosomal subunit
Very long chain specific acyl coenzyme A dehydrog enase	**A0A077ZIT9**	10	64	-	acyl-CoA dehydrogenase	-	-
HSP70 and LRR 8 and LRR 1 domain containing prote in	**A0A077ZB90**	10	57	-	ATP-dependent protein folding chaperone	-	-
Multifunctional protein ADE2	**A0A077ZCJ9**	10	48	-	phosphoribosylaminoimidazole succinocarboxamide synthase activity	'de novo' IMP biosynthetic process	-
Protein EMB 9 a	**A0A077YZN4**	9	171	-	extracellular matrix structural constituent	anatomical structure development	membrane

VAB 10A protein	**A0A077ZB12**	9	855	-	actin binding	intermediate filament cytoskeleton organization	cytoskeleton
TBP and UBA and ubiquitin domain containing prote in	**A0A077Z006**	9	82	-	DNA binding	DNA-templated transcription initiation	-
Ribos L4 asso C and Ribosomal L4 domain containin g protein	**A0A077Z5S6**	9	44	-	structural constituent of ribosome	translation	ribosome
Uncharacterized protein	**A0A077Z0J7**	9	44	-	-	-	-
Nucleosome assembly protein 1 1	**A0A077YWU6**	9	38	-	-	nucleosome assembly	nucleus
DeoC and LMWPc domain containing protein	**A0A077ZCG9**	9	63	-	protein tyrosine phosphatase activity	protein dephosphorylation	cytoplasm
LIM domain containing protein	**A0A077Z1J3**	8	39	-	metal ion binding	-	-
NADH dehydrogenase [ubiquinone] flavoprotein 1 mitochondrial	**A0A077YXA9**	8	54	-	NADH dehydrogenase (ubiquinone) activity	-	respiratory chain complex I
Angiotensin-converting enzyme	**A0A077YZS2**	8	206	-	metallopeptidase activity	proteolysis	membrane
40S ribosomal protein S6	**A0A077ZFJ1**	8	26	-	structural constituent of ribosome	translation	ribosome
Prohibitin protein WPH	**A0A077ZCU4**	8	30	-	mitochondrial inner membrane	-	-
AP complex subunit beta	**A0A077ZF94**	8	102	-	clathrin binding	intracellular protein transport	clathrin adaptor complex
Acyl-CoA synthetase family membe 2 mitochondria l	**A0A077ZH85**	8	95	-	-	-	-
Uncharacterized protein	**A0A077YXJ4**	8	16	-	-	-	-
Uncharacterized protein	**A0A077Z0U1**	7	285	-	-	-	-
I-set and fn3 and Ig 2 domain containing protein	**A0A077ZD63**	7	93	01-21	-	cell adhesion	membrane
Putative calcium-dependent protein kinase family protein	**A0A077Z8E2**	7	26	-	kinase activity	phosphorylation	-
Amidinotransf domain containing protein	**A0A077YXL4**	7	29	-	dimethylargininase activity	-	-
1-cysPrx C and AhpC-TSA domain containing protein	**A0A077ZCA9**	7	24	-	peroxiredoxin activity	-	-

Integrin beta	**A0A077Z8U9**	7	89	-	-	integrin-mediated signaling pathway	membrane
Sodium:potassium transporting atpase subunit	**A0A077YZ37**	7	34	-	-	sodium ion transport	sodium:potassium-exchanging ATPase complex
Beta parvin	**A0A077YXM0**	7	42	-	actin binding	actin cytoskeleton reorganization	cytoskeleton
Ribosomal protein S3	**A0A077Z7F8**	6	23	-	RNA binding	translation	small ribosomal subunit
Isochorismatase domain containing protein	**A0A077Z389**	6	23	-	-	-	-
Transmembrane cell adhesion receptor mua 3	**A0A077Z1A0**	6	286	-	calcium ion binding	-	-
T-complex protein 1 subunit gamma	**A0A077Z2L0**	6	60	-	ATP-dependent protein folding chaperone	-	chaperonin-containing T-complex
La protein	**A0A077YZD7**	6	50	-	RNA binding	RNA processing	nucleus
DUF1679 and EcKinase domain containing protein	**A0A077Z471**	6	209	-	protein kinase activity	protein phosphorylation	-
Glyco hydro 18 domain containing protein	**A0A077Z380**	6	37	01-21	chitin binding	carbohydrate metabolic process	-
Solute carrier family 2 facilitated glucose	**A0A077Z5C9**	6	56	-	transmembrane transporter activity	-	membrane
Histidine triad nucleotide binding protein 1	**A0A077YVI5**	6	14	-	catalytic activity	-	-
60S ribosomal protein L9	**A0A077ZF43**	6	21	-	rRNA binding	translation	ribosome
Acid ceramidase	**A0A077Z1B3**	6	45	01-21	fatty acid amide hydrolase activity	fatty acid metabolic process	lysosome
Uncharacterized protein	**A0A077YZJ9**	6	25	01-17	-	-	-
Glyco hydro 19 domain containing protein (Fragment)	**A0A077ZLY8**	6	38	-	chitinase activity	chitin catabolic process	-
Endostatin domain containing protei	**A0A077Z4M2**	6	16	-	-	-	-
E1 DerP2 DerF2 domain containing protein	**A0A077ZES0**	6	17	-	-	-	-
40S ribosomal protein S8 (Fragment	**A0A077YZU1**	6	25	-	structural constituent of ribosome	translation	ribosome

E1 DerP2 DerF2 domain containing protein	**A0A077Z3A9**	5	35	-	sterol binding	sterol transport	-
Arginine kinase	**A0A077Z8H1**	5	42	-	ATP binding	phosphorylation	-
Endophilin protein 1 b	**A0A077ZC07**	5	31	-	-	-	Golgi apparatus
TPR 11 domain containing protein	**A0A077Z8W3**	5	17	-	-	-	-
Acetyl coenzyme A acetyltransferas mitochondria l	**A0A077Z8S0**	5	43	-	acyltransferase activity	-	-
Porin 3 domain containing protein	**A0A077Z742**	5	24	-	porin activity	-	pore complex
Embryonic fatty acid-binding protei Bm-FAB-1	**A0A077YX80**	5	44	-	-	-	-
Ldl recept a and CUB and Trypsin domain containin g protein	**A0A077ZDJ0**	5	75	01-21	serine-type endopeptidase activity	proteolysis	-
Neurogenic locus notch protein 2	**A0A077ZG34**	5	92	01-25	calcium ion binding	-	membrane
Uncharacterized protein	**A0A077Z5V8**	5	20	-	-	-	-
Serine:threonine protein phosphatas 5	**A0A077YXQ6**	5	58	-	myosin phosphatase activity	-	cytoplasm
40S ribosomal protein S16	**A0A077ZGK9**	5	18	-	structural constituent of ribosome	translation	ribosome
Udp glucose:glycoprotein glucosyltransferase	**A0A077YXS6**	5	188	01-31	UDP-glucose:glycoprotein glucosyltransferase activity	protein glycosylation	endoplasmic reticulum lumen
Carboxypeptidase e	**A0A077Z8Q4**	5	173	01-24	carboxypeptidase activity	-	membrane
E1 DerP2 DerF2 domain containing protein	**A0A077ZIB9**	5	17	-	-	-	-
FERM N domain containing protein	**A0A077Z9Z6**	5	13	-	actin binding	-	cytoskeleton
Tropomodulin family protein	**A0A077Z401**	5	48	-	tropomyosin binding	pointed-end actin filament capping	cytoskeleton
ADP ribosylation factor 1	**A0A077ZIF5**	5	87	-	GTPase activity	protein transport	Golgi apparatus
Protein TFG	**A0A077Z1I2**	5	48	-	identical protein binding	COPII vesicle coating	cytoplasm
Ribokinase	**A0A077ZI00**	4	33	-	ribokinase activity	D-ribose catabolic proces	nucleus
Laminin protein lam 2	**A0A077Z4D6**	4	181	-	-	-	-
EF-hand 7 domain containing protei	**A0A077ZHV0**	4	23	-	calcium ion binding	-	-

Lactoylglutathione lyase	**A0A077Z5J4**	4	20	-	lactoylglutathione lyase activity	-	-
60S ribosomal protein L8	**A0A077ZC94**	4	29	-	RNA binding	translation	large ribosomal subunit
Acetylcholinesterase	**A0A077Z8B4**	4	164	-	acetylcholinesterase activity	neurotransmitter catabolic process	-
Chloride intracellular channel exc 4	**A0A077Z6X1**	4	34	-	-	-	-
Phosphotransferase	**A0A077Z3Y9**	4	55	-	glucokinase activity	glycolytic process	-
Beta-galactosidase	**A0A077Z195**	4	72	-	beta-galactosidase activity	carbohydrate metabolic process	lysosome
Cytosol aminopeptidase	**A0A077Z7G6**	4	48	-	metalloaminopeptidase activity	proteolysis	cytoplasm
Transcriptional activator protein Pur alpha	**A0A077Z9C0**	4	30	-	RNA polymerase II transcription regulatory region sequence-specific DNA binding	-	nucleus
Succinate dehydrogenase cytochrom b560 subunit	**A0A077YWQ6**	4	20	-	succinate dehydrogenase activity	tricarboxylic acid cycle	succinate dehydrogenase complex
Malectin domain containing protein	**A0A077Z9Z2**	4	30	01-23	carbohydrate binding	-	endoplasmic reticulum membrane
Uncharacterized protein	**A0A077ZC88**	4	41	01-24	-	-	-
Prostatic acid phosphatase lysosoma acid phosph atase	**A0A077YZX7**	3	36	-	-	-	-
Reticulon-like protein	**A0A077Z2C5**	3	23	-	-	-	endoplasmic reticulum membrane
Hexosyltransferase	**A0A077ZFU3**	3	125	01-17	acetylgalactosaminyltransferase activity	-	Golgi cisterna membrane
Uncharacterized protein	**A0A077ZFW7**	3	16	-	-	-	-
Coatomer subunit epsilon	**A0A077ZBE0**	3	34	-	structural molecule activity	retrograde vesicle-mediated transport	Golgi membrane
Ubiquitin carboxyl terminal hydrolase 7	**A0A077ZB91**	3	81	-	cysteine-type deubiquitinase activity	ubiquitin-dependent protein catabolic process	-
Ribosomal protein L24	**A0A077Z4E8**	3	21	-	-	-	ribosome
PALP domain containing protein	**A0A077ZG83**	3	41	-	-	-	-
Calcyclin binding protein	**A0A077YZA1**	3	25	-	ubiquitin protein ligase binding	-	-

Zf-CDGSH domain containing protein	**A0A077Z600**	3	10	-	metal ion binding	regulation of autophagy	cytoplasm
Uncharacterized protein	**A0A077YZF3**	3	41	01-16	-	-	-
TIA 1 ue	**A0A077ZER1**	3	35	-	RNA binding	-	-
HEGF and CUB domain containing protein	**A0A077ZAT4**	3	100	-	calcium ion binding	-	membrane
Stomatin protein 2	**A0A077ZD38**	3	37	-	-	-	membrane
26S proteasome non-ATPase regulatory subunit 8	**A0A077ZER9**	3	32	-	-	proteolysis	proteasome regulatory particle
Adenylyl cyclase-associated protein	**A0A077Z7C2**	3	64	-	actin binding	cytoskeleton organization	-
Uncharacterized protein	**A0A077ZLP6**	3	36	-	-	-	-
Methylthioribose-1-phosphate isomerase	**A0A077ZAX0**	3	40	-	S-methyl-5-thioribose-1-phosphate isomerase activity	L-methionine salvage from methylthioadenosine	nucleus
Pyridoxal deC domain containing protein	**A0A077Z7B3**	3	55	-	carbon-carbon lyase activity	carboxylic acid metabolic process	-
Uncharacterized protein	**A0A077YXJ5**	3	17	-	-	-	-
Mannose 1 phosphate guanyltransferase beta	**A0A077YY15**	3	42	-	mannose-1-phosphate guanylyltransferase (GTP) activity	GDP-mannose biosynthetic process	-
cAMP-dependent protein kinase regulatory subunit	**A0A077ZK85**	3	28	-	kinase activity	phosphorylation	-
Peroxidasin	**A0A077Z1Z5**	3	154	01-24	lactoperoxidase activity	response to oxidative stress	extracellular region
Uncharacterized protein	**A0A077ZJU5**	3	49	-	-	-	-
60S ribosomal protein L23a (Fragment)	**A0A077ZHL7**	3	21	-	structural constituent of ribosome	translation	ribosome
Uncharacterized protein	**A0A077YYW5**	3	42	-	-	-	-
Uncharacterized protein	**A0A077Z2A1**	3	16	-	-	-	membrane
Angiotensin-converting enzyme	**A0A077YXE5**	3	67	01-19	peptidyl-dipeptidase activity	proteolysis	membrane
CUB and EGF domain containing protein	**A0A077ZPX9**	2	35	-	calcium ion binding	-	-
Peptidase M16 C and Peptidase M16 domain containi ng protein	**A0A077Z262**	2	46	-	metalloendopeptidase activity	proteolysis	mitochondrial matrix

Histidyl tRNA synthetase cytoplasmic	**A0A077ZI64**	2	50	-	histidine-tRNA ligase activity	-	cytoplasm
LRR 5 domain containing protein	**A0A077Z094**	2	46	01-28	-	-	-
Proteasome endopeptidase complex	**A0A077ZAW8**	2	27	-	-	proteolysis involved in protein catabolic process	nucleus
Reticulon-like protein	**A0A077Z7P1**	2	68	-	-	-	endoplasmic reticulum membrane
Glyco hydro 19 domain containing protein	**A0A077ZFP5**	2	34	-	chitinase activity	chitin catabolic process	-
Ubiquinol cytochrome c reductase cytochrome c1	**A0A077ZAF0**	2	30	-	electron transfer activity	-	mitochondrial inner membrane
Barrier to autointegration factor	**A0A077ZFN1**	2	10	-	DNA binding	-	nucleus
SH3 9 and SH3 1 domain containing protein	**A0A077Z5Y9**	2	98	-	-	-	cytoplasm
Mediator of RNA polymerase II transcription subun it 22	**A0A077ZPP8**	2	17	-	-	-	membrane
NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	**A0A077ZKF4**	2	21	-	-	mitochondrial electron transport	mitochondrial inner membrane
L51 S25 CI-B8 domain containing protein	**A0A077Z9G5**	2	19	-	-	-	mitochondrial inner membrane
I-set domain containing protein	**A0A077Z8Q3**	2	41	01-19	-	cell adhesion	plasma membrane
Cyt-b5 domain containing protein	**A0A077ZJT8**	2	12	-	metal ion binding	-	-
Dynein light chain	**A0A077ZJN3**	2	10	-	-	microtubule-based process	microtubule
Hypoxanthine phosphoribosyltransferase	**A0A077ZBM1**	2	25	-	nucleotide binding	purine ribonucleoside salvage	cytoplasm
RRM 1 domain containing protein	**A0A077ZD02**	2	52	-	RNA binding	-	-
ShK domain containing protein	**A0A077Z795**	2	26	-	-	-	-
Peptidase S28 domain containing protein	**A0A077ZCE4**	2	51	01-20	serine-type peptidase activity	proteolysis	-
Lysosomal acid phosphatase	**A0A077Z586**	2	45	-	-	-	membrane
Calreticulin domain containing protein	**A0A077YYB3**	2	82	01-16	unfolded protein binding	protein folding	endoplasmic reticulum
ATP binding cassette sub family F	**A0A077Z9Q1**	2	73	-	ATP hydrolysis activity	-	-

Uncharacterized protein	**A0A077ZDG2**	2	20	-	-	-	-
26S proteasome non ATPase regulatory subunit 14	**A0A077ZKC9**	2	34	-	metallopeptidase activity	-	proteasome complex
Pregnancy-associated glycoprotein 1	**A0A077Z943**	2	68	01-24	aspartic-type endopeptidase activity	proteolysis	-
Uncharacterized protein	**A0A077ZCC4**	2	24	-	-	-	membrane
Phosphorylase b kinase regulatory subunit	**A0A077Z722**	2	147	-	kinase activity	phosphorylation	plasma membrane
Peptidase C12 and PRP38 and CRAL TRIO domain cont aining protein	**A0A077Z5Z3**	2	126	-	cysteine-type deubiquitinase activity	ubiquitin-dependent protein catabolic process	spliceosomal complex

a: Protein accession number in accordance with the UniProt database.

b: Gene Ontology (GO) Annotations in accordance with the software Omixbox database.

Proteins that obtained at least 2 peptides identified by LC-MS/MS are described in the table. Preoteins with lower scores are not presented.

Table 03. Proteins identified in Trichuris trichiura Female extract proteome by LC-MS/MS using Proteome Discoverer Version 1.4 (Thermo Fisher Scientific) with SequestHT as the search engine and PEAKS Studio 8 (Bioinformatics Solutions, Waterloo, ON, Canada). and OmixBox Ontology database.

Description	Accession number[a]	Peptides	MW (kDa)	Signal Peptide	Molecular function[b]	Biological Process[b]	Cellular component[b]
Vitellogenin N and VWD and DUF1943 domain contain ing protein	**A0A077ZE83**	231	199	01-19	lipid transporter activity		
TIL and CBM 14 domain containing protein	**A0A077ZFY4**	23	196	01-31	chitin binding		
Tyrosinase domain containing protein	**A0A077Z814**	23	53	01-19	oxidoreductase activity		
CBM 14 domain containing protein	**A0A077Z8B3**	14	79	-	chitin binding		extracellular region
Uncharacterized protein	**A0A077Z0R1**	12	18	01-19			
Bifunctional purine biosynthesis protein	**A0A077Z6Z8**	8	66	-	Transferase	Purine biosynthesis	
TSP 1 and CBM 14 domain containing protein	**A0A077Z3K5**	8	93	-	chitin binding		extracellular region
Uncharacterized protein	**A0A077YXT2**	6	70	01-18			
Cadherin C and Laminin G 2 and Cadherin domain co ntaining protein	**A0A077Z8V8**	6	287	-	calcium ion binding	morphogenesis of an epithelium	plasma membrane
Cpn10 domain containing protein	**A0A077ZE65**	5	10	-	ATP-dependent protein folding chaperone		
CBM 14 domain containing protein	**A0A077ZFK2**	5	192	-	chitin binding		extracellular region
HSP20 domain containing protein	**A0A077Z8Q1**	5	12	-			
Uncharacterized protein	**A0A077Z453**	4	172	-			
Ribosomal protein L15	**A0A077YZY9**	4	29	01-16			ribosome
Uncharacterized protein (Fragment)	**A0A077ZIB0**	4	41	-			
StAR-related lipid transfer protein 5	**A0A077YVV8**	4	28	-	lipid binding		
Kunitz protease inhibitor	**A0A077YUJ5**	4	27	01-18	serine-type endopeptidase inhibitor activity		
Chymotrypsin-elastase inhibitor ixodidin	**A0A077Z7B4**	4	10	-	Serine protease inhibitor	negative regulation of peptidase activity	extracellular region
Uridine phosphorylase 1	**A0A077Z178**	3	69	-	uridine phosphorylase activity	nucleotide catabolic	cytoplasm

SecE and NusG and KOW and Ribosomal L11 N and Rib osomal L11 domain containing protein	**A0A077ZH59**	2	45	-	structural constituent of ribosome	Translation	ribosome
Histidine acid phosphatase family protein	**A0A077Z7Y2**	2	48	-			
Putative casein kinase substrate phosphoprotein p p28	**A0A077ZBM2**	2	23	-	kinase activity	phosphorylation	
Uncharacterized protein	**A0A077Z2P1**	2	19	01-16			
Charged multivesicular body protein 2a	**A0A077Z572**	2	25	-		vacuolar transport	
RNA polymerase II transcriptional coactivator	**A0A077Z9X8**	2	12	-	transcription coactivator activity	positive regulation of transcription initiation by RNA polymerase II	nucleus
Uncharacterized protein	**A0A077Z055**	2	14	-			
Peptidase C1 domain containing protein	**A0A077Z108**	2	28	-	cysteine-type peptidase activity	Proteolysis	
Thioredoxin and ATP-synt ab and Rho N and Rho RNA bind domain containing protein	**A0A077ZDQ6**	2	59	-	RNA binding	DNA-templated transcription termination	
PNP UDP 1 domain containing protein	**A0A077ZCC7**	2	26	-	Glycosyltransferase	nucleoside catabolic process	
DUF788 and Tsg domain containing protein	**A0A077YZ91**	2	45	01-25			extracellular region
Uncharacterized protein	**A0A077Z8E8**	2	22				

a: Protein accession number in accordance with the UniProt database.

b: Gene Ontology (GO) Annotations in accordance with the software Omixbox database.

Proteins that obtained at least 2 peptides identified by LC-MS/MS are described in the table. Preoteins with lower scores are not presented.

Table supplementary 4: Proteins Uncharacterized protein identified in Trichuris trichiura adult worms extract proteome by LC-MS/MS using Proteome Discoverer Version 1.4 (Thermo Fisher Scientific) with SequestHT as the search engine and PEAKS Studio 8 (Bioinformatics Solutions, Waterloo, ON, Canada). and OmixBox Ontology database.

Description	Accession number[a]	Peptides	MW (kDa)	Signal Peptide	Molecular function[b]	Biological Process[b]	Cellular component[b]
Uncharacterized protein	**A0A077Z544**	65	34	01-23	-	-	-
Uncharacterized protein	**A0A077YYL1**	52	28	-	-	-	-
Uncharacterized protein	**A0A077YZI4**	31	99	-	-	-	-
Uncharacterized protein	**A0A077Z373**	22	34	01-20	-	-	-
Uncharacterized protein	**A0A077YW28**	20	36	01-22	-	-	-
Uncharacterized protein	**A0A077Z8F0**	16	31	-	-	-	-
Uncharacterized protein	**A0A077Z6A6**	14	107	01-19	-	-	membrane
Uncharacterized protein	**A0A077Z482**	12	187	01-18	calcium ion binding	-	membrane
Uncharacterized protein	**A0A077ZI12**	10	58	-	-	-	-
Uncharacterized protein	**A0A077ZCJ0**	10	12	-	-	-	-
Uncharacterized protein	**A0A077YZV0**	9	22	-	-	-	-
Uncharacterized protein	**A0A077YVT6**	9	15	-	-	-	-
Uncharacterized protein	**A0A077YYK9**	9	17	-	-	-	-
Uncharacterized protein	**A0A077Z9S1**	8	96	-	-	-	-
Uncharacterized protein	**A0A077Z0L5**	8	119	01-22	-	-	-
Uncharacterized protein	**A0A077ZAZ5**	8	58	01-24	-	-	-
Uncharacterized protein	**A0A077YZ75**	6	14	-	-	-	-
Uncharacterized protein	**A0A077ZKJ0**	5	65	-	-	-	-
Uncharacterized protein	**A0A077ZE99**	5	135	-	-	-	-
Uncharacterized protein	**A0A077Z7J9**	3	132	01-25	-	-	-
Uncharacterized protein	**A0A077ZIV9**	3	132	-	-	-	-
Uncharacterized protein	**A0A077ZGV8**	3	19	-	-	-	-
Uncharacterized protein	**A0A077ZK49**	3	14	01-19	-	-	-
Uncharacterized protein	**A0A077ZGB3**	2	32	01-20	-	-	

Uncharacterized protein	**A0A077Z4J9**	2	26	-	-	-	endosome membrane
Uncharacterized protein	**A0A077YZ92**	2	65	-	-	-	-
Uncharacterized protein	**A0A077Z0R1**	12	18	01-19			
Uncharacterized protein	**A0A077YXT2**	6	70	01-18			
Uncharacterized protein	**A0A077Z453**	4	172	-			
Uncharacterized protein (Fragment)	**A0A077ZIB0**	4	41	-			
Uncharacterized protein	**A0A077Z2P1**	2	19	01-16			
Uncharacterized protein	**A0A077Z055**	2	14	-			
Uncharacterized protein	**A0A077Z8E8**	2	22				
Uncharacterized protein	A0A077Z0M1	11	19	-	-	-	-
Uncharacterized protein	**A0A077Z0J7**	9	44	-	-	-	-
Uncharacterized protein	**A0A077YXJ4**	8	16	-	-	-	-
Uncharacterized protein	**A0A077Z0U1**	7	285	-	-	-	-
Uncharacterized protein	**A0A077YZJ9**	6	25	01-17	-	-	-
Uncharacterized protein	**A0A077Z5V8**	5	20	-	-	-	-
Uncharacterized protein	**A0A077ZC88**	4	41	01-24	-	-	-
Uncharacterized protein	**A0A077ZFW7**	3	16	-	-	-	-
Uncharacterized protein	**A0A077YZF3**	3	41	01-16	-	-	-
Uncharacterized protein	**A0A077ZLP6**	3	36	-	-	-	-
Uncharacterized protein	**A0A077YXJ5**	3	17	-	-	-	-
Uncharacterized protein	**A0A077ZJU5**	3	49	-	-	-	-
Uncharacterized protein	**A0A077YYW5**	3	42	-	-	-	-
Uncharacterized protein	**A0A077Z2A1**	3	16	-	-	-	membrane
Uncharacterized protein	**A0A077ZDG2**	2	20	-	-	-	-
Uncharacterized protein	**A0A077ZCC4**	2	24	-	-	-	membrane

a: Protein accession number in accordance with the UniProt database.

b: Gene Ontology (GO) Annotations in accordance with the software Omixbox database.

Proteins that obtained at least 2 peptides identified by LC-MS/MS are described in the table. Preoteins with lower scores are not presented.

6. CONCLUSÕES E CONSIDERAÇÕES FINAIS

O parasito *Trichuris trichiura* continua a representar um desafio significativo em termos de saúde pública em todo o mundo, com morbidade considerável e alta carga global de doença (CALDRER; URSINI; SANTUCCI; MOTTA *et al.*, 2022). Os que sofrem mais intensamente com a infecção são geralmente indivíduos em situações de pobreza em países de baixa e media renda, onde o acesso limitado ao saneamento básico, sistemas de saúde inadequados e a falta de uma vigilância epidemiológica eficaz criam um ambiente propício para a disseminação da infecção e o agravamento dos sintomas. Além disso, nessas áreas com recursos limitados, o diagnóstico precoce e o tratamento adequado da infecção podem ser escassos, o que favorece a disseminação do parasito e aumento dos casos de reinfecções, tornando assim mais difícil o controle efetivo da tricuríase (GANGULY; BARKATAKI; SANGA; BOOPATHI *et al.*, 2022; LOUKAS; MAIZELS; HOTEZ, 2021).

Embora *T. trichiura* represente um problema de saúde pública de altas proporções, a longa convivência com seu hospedeiro humano desencadeou uma espécie de "corrida armamentista" na evolução, na qual o parasito desenvolveu estratégias para sobreviver ao ambiente hostil do seu hospedeiro por meio da produção de moléculas bioativas, principalmente proteínas, responsáveis por modular a resposta imune do parasitado a seu favor, adaptando a resposta ao parasitismo (MAIZELS; SMITS; MCSORLEY, 2018; STEVENSON; VALANPARAMBIL; TAM, 2022).

Essa interação contínua gerou um rico tesouro de oportunidades ainda não totalmente exploradas que podem ter implicações significativas na medicina, principalmente em doenças alérgicas, inflamatórias, e autoimune (GONCALES; NOBREGA; NASCIMENTO; LORENA *et al.*, 2020; LOUKAS; MAIZELS; HOTEZ, 2021; MUGHAL; KHAN; ABBAS; ABBAS *et al.*, 2021). Deste modo, o entendimento dos processos utilizados pelo *T. trichiura* em seus diferentes estágios de vida pode gerar melhor entendimento da sua biologia, produzir informações úteis em diversos campos da saúde pública (CRUZ; MARCILLA; KELLY; VANDENPLAS *et al.*, 2021).

A análise proteômica, fundamentada na espectrometria, desempenha um papel significativo na ampliação do conhecimento relacionado à complexa interação entre parasitos helmintos e seus hospedeiros. Ela tem se estabelecido como uma ferramenta essencial na busca por moléculas derivadas desses parasitos que possam ter aplicabilidade no desenvolvimento de novos métodos diagnósticos, terapias inovadoras e medidas preventivas de natureza imunológica (ROBINSON; CWIKLINSKI, 2021).

Embora a espectrometria de massa (MS) já seja uma ferramenta de diagnóstico amplamente utilizada na microbiologia clínica há algum tempo, seu uso como meio de diagnóstico para espécies adultas de *Trichuris* só foi recentemente descrito (RIVERO; ZURITA; CUTILLAS; CALLEJON, 2022). Outros trabalhos também têm evidenciado a eficácia da MS como ferramenta diagnóstica na helmintologia humana e veterinária, sugerindo um potencial significativo para a identificação confiável de parasitos como *Trichinella spp, Caenorhabditis elegans* e *Dirofilaria spp* (FEUCHEROLLES; POPPERT; UTZINGER; BECKER, 2019).

O proteoma de diversos helmintos tem demonstrado a diversidade proteica desses parasitos em suas diferentes fases de vida, assim como em seus produtos excretados e secretados, que exibem um potencial considerável na modulação da resposta imune. (SOTILLO; ROBINSON; KIMBER; CUCHER *et al.*, 2020). A. suum adulto e em seu estágio larval L4 (HANSEN; FROMM; ANDERSEN; MARCILLA *et al.*, 2019), *Nippostrongylus brasiliensis* e *T. muris* adultos (EICHENBERGER; RYAN; JONES; BUITRAGO *et al.*, 2018; WANGCHUK; KOUREMENOS; EICHENBERGER; PEARSON *et al.*, 2019), tegumento do verme adulto *S. mansoni* (PEARSON; LOUKAS; SOTILLO, 2020) e miracídio (WANG; ZHAO; ROTGANS; STRONG *et al.*, 2016) e extratosomático de *Schistosoma mekongi* adulto (REAMTONG; SIMANON; THIANGTRONGJIT; LIMPANONT *et al.*, 2020) são exemplos que revelam o potencial da análise proteômica na compreensão da diversidade proteica de helmintos e suas implicações na modulação da resposta imune.

Avaliações com *T. trichiura* já demostraram que tanto o proteoma dos ovos do parasito quanto seus produtos excretados/secretados possuem presença de uma variedade de proteínas com potencial imunomodulador (AGUIAR-SANTOS; MEDEIROS; BONFIM; ROCHA *et al.*, 2013; CRUZ; MARCILLA; KELLY; VANDENPLAS *et al.*, 2021). A análise por nós realizada do proteoma integral do parasito adulto macho e fêmea proporciona a oportunidade de aprofundar nosso entendimento da biologia desse parasito e identificar potenciais moléculas imunomoduladoras.

No estudo citado, o proteoma integral do parasito *T. trichiura* macho e fêmea foi analisado por LC-MS/MS, juntamente com o uso de diversas ferramentas de bioinformática. Essa abordagem possibilitou a identificação de quantidade significativa de proteínas, resultados compatíveis com as previsões do transcriptoma (SANTOS; SILVA; SANTOS; DE SA *et al.*, 2016) e a classificação entre proteínas compartilhadas entre machos e fêmeas, exclusivas por gênero e uma diversidade de proteínas classificadas como não

caracterizadas, que foram localizadas nesse proteoma. Fato essencial para determinação da existência dessas proteínas, uma vez que os transcritos foram anotados como proteínas hipotéticas.

Ontologia genética foi empregada para avaliar o perfil de cada grupo (macho integral e somente as proteínas exclusivas, fêmea integral e somente as proteínas exclusivas e proteínas compartilhadas entre os gêneros). Isso possibilitou a identificação de grupos proteicos com seus respectivos componentes, funções e processos, contribuindo para um melhor entendimento da biologia do parasito.

Com a produção da proteína rc4299 e sua avaliação em PBMCs foi possível comprovar seu perfil imunorreativo e imunomodulador. Isso abre perspectivas futuras para o seu uso, tanto de forma isolada quanto em consórcio com fármacos e no tratamento de doenças inflamatórias. Além disso, este trabalho descreve diversas proteínas presentes no proteoma de *T. trichiura* com potencial imunomodulador, como a inibidora de protease Kunitz, que são inibidoras de serino protease bem conhecidas por ter como alvo proteases essenciais envolvidas na inflamação. A proteína mencionada é apenas uma das várias identificadas apenas nesse trabalho, em comparação com o proteoma do extrato proteico e dos ovos de *T. trichiura* (AGUIAR-SANTOS; MEDEIROS; BONFIM; ROCHA *et al.*, 2013; CRUZ; MARCILLA; KELLY; VANDENPLAS *et al.*, 2021).

O trabalho apresentado aqui lança luz sobre diversos processos e evidencia as distinções moleculares entre o parasito macho e fêmea do verme adulto *T. trichiura*. Além disso, demonstra que proteínas derivadas do parasito desempenham, de fato, um papel imunomodulador, como exemplificado pela proteína rc4299. O estudo também fornece uma lista de proteínas de *T. trichiura*, segregadas por gênero, que podem ser usadas em pesquisas futuras para entender a patogênese da tricuríase humana, na bioprospecção de medicamentos para controle da tricuríase, bem como para a produção de imunobiológicos e moléculas úteis em doenças alérgicas, inflamatórias e autoimunes.

7 PERSPECTIVAS FUTURAS

- Realizar mais estudos com o objetivo de viabilizar o uso da proteína rc4299 no tratamento de doenças inflamatórias.
- Identificar, nos dados do proteoma e por meio de análises *in silico*, moléculas com potencial para o diagnóstico da tricuríase, bem como moléculas viáveis para imunoprofilaxia.
- Utilizar o proteoma gerado neste trabalho para análises *in sílico* e in vitro a fim de realizar o reposicionamento de fármacos.

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9 APÊNDICE

9.1 PATENTES PUBLICADAS DURANTE O DOUTORADO

Patente BR 10 2019 021511 9 - Submetida ao INPI
Data do Depósito: 14/10/2019
Data da Publicação Nacional: 17/08/2021
Participação como **inventor colaborador/coautor**

Título: Método de produção de proteínas recombinantes do *Toxocara canis*, visadas como vacina para controle da toxocaríase canina"

Patente BR 10 2020 024449 3 - Submetida ao INPI
Data do Depósito: 30/11/2020
Data da Publicação Nacional: 14/06/2022
Participação como **inventor colaborador/coautor**

Título: Processo de construção e produção de proteínas recombinants hipoalergênicas para uso profilático e terapêutico de doenças alérgicas causadas pelo ácaro da poeira doméstica *Dermatophagoides pteronyssinus*.

9.2 ARTIGOS PUBLICADAS DURANTE O DOUTORADO

Título	Revista	DOI	Ano de publicaçã o	Autoria
The proteome of the adult human whipworm Trichuris trichiura: a source of potential immunomodulator y molecules	Biochimica et Biophysica Acta Journal: (BBA) - Proteins and Proteomics	Manuscript Number: BBAPRO-23-204	Submetido em 2023	Primeiro autor
The hybrid protein BTH2 suppresses allergic airway inflammation in a murine model of HDM- specific Immunotherapy	Clinical & Experimental Allergy	10.1111/cea.14293	2023	Coautor
Recombinant T-cell epitope conjugation: A new approach for	Clinical & Experimental Allergy	10.1111/cea.14238	2023	Coautor

Dermatophagoides hypoallergen design				
Engineering an optimized expression operating unit for improved recombinant protein production in Escherichia coli	Protein Expression and Purification	10.1016/j.pep.2022.106150	2022	Coautor
Proteomics and immunoblotting analyses reveal antigens that optimize the immunodiagnosis of the infection by Toxocara spp	Transboundary and Emerging Diseases	10.1111/tbed.14650	2022	Coautor
Identification of Glycycometus malaysiensis (for the first time in Brazil), Blomia tropicalis and Dermatophagoides pteronyssinus through multiplex PCR	Experimental and Applied Acarology	10.1007/s10493-022-00694-y	2022	Coautor
Immunomodulatory properties of Schistosoma mansoni proteins Sm200 and SmKI-1 in vitro and in a murine model of allergy to the mite Blomia tropicalis	Molecular Immunology	10.1016/j.molimm.2020.05.011	2020	Coautor
Immunogenicity and protection induced by recombinant Toxocara canis proteins in a murine model of Toxocariasis	Vaccine	10.1016/j.vaccine.2020.04.072	2020	Coautor