UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

PATRONES DE DIVERSIDAD DE PARÁSITOS EN CÍCLIDOS DE LOS LAGOS DE NICARAGUA

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

ANA OFELIA SANTACRUZ VÁZQUEZ

TUTOR PRINCIPAL DE TESIS: DR. GERARDO PÉREZ-PONCE DE LEÓN, INSTITUTO DE BIOLOGÍA, UNAM

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TUTOR INVITADO: DRA. MARTA BARLUEGA BADIOLA, MUSEO NACIONAL DE CIENCIAS NATURALES, CSIC

Ciudad Universitaria, CD. MX. **2022**

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POSGRADO CIENCIAS BIOLÓGICAS

COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE BIOLOGÍA
OFICIO CPCB/036/2022
ASUNTO: Oficio de Jurado

M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
Presente

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **22 de noviembre de 2021** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **SANTACRUZ VÁZQUEZ ANA OFELIA** con número de cuenta **515015531** con la tesis titulada **“PATRONES DE DIVERSIDAD DE PARÁSITOS EN CÍCLIDOS DE LOS LAGOS DE NICARAGUA”,** realizada bajo la dirección del **DR. GERARDO PÉREZ PONCE DE LEÓN,** quedando integrado de la siguiente manera:

Presidente: DR. ROGELIO AGUILAR AGUILAR
Vocal: DR. JUAN JOSÉ MORRONE
Vocal: DR. ALEJANDRO EMMANUEL GONZÁLEZ VOYER
Vocal: DRA. VIRGINIA LEÓN REGAGNON
Secretario: DRA. CLAUDIA PATRICIA ORNELAS GARCÍA

Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
“POR MI RAZA HABLARÁ EL ESPÍRITU”
Ciudad Universitaria, Cd. Mx., a 13 de enero de 2022

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

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Ma famille, mes amis, mes amours.

Dedicatoria

Dedico este estudio a mi padre,
Darwin & Bob

"*There is grandeur in this view of life,…whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved".*

Darwin

When you hold a bird (fish) in your hand,
you are essentially holding the parasite's niche.

Dale H. Cleyton

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RESUMEN

Hospederos y parásitos están involucrados en interacciones complejas de constante adaptación recíproca, imponiendo una fuerte presión de selección capaz de alterar sus trayectorias evolutivas. Los hospederos colonizan nuevos ambientes con un subconjunto de sus parásitos originales, pudiendo perder parásitos y formando nuevos ensambles. La exposición de los hospederos a diferentes comunidades de parásitos puede suponer presiones de selección distintas entre las poblaciones que disparen o aceleren la divergencia. El cíclido de Midas es un modelo extraordinario para estudiar los mecanismos que impulsan la especiación simpátrica y alopátrica, y la evolución de paralelismos. El escenario geográfico para su evolución son los lagos grandes y lagos cráter de Nicaragua, un escenario análogo a un modelo continente-isla. El marco ecológico replicado en diversos lagos y la variación temporal de su evolución dentro de los lagos cráter, además de la especiación reciente o en curso, provee un panorama de las condiciones iniciales que dieron lugar a la divergencia, y que permite aproximarse a los mecanismos que guían la divergencia en un continuo de especiación.

En este estudio investigamos el rol de la selección mediada por parásitos en las radiaciones adaptativas y en paralelo del cíclido de Midas. Si las poblaciones de hospederos están expuestas a distintas comunidades de parásitos, y son diferencias temporalmente estables, esto podría promover la diversificación. Para ello, estudiamos a lo largo de tres años consecutivos las comunidades de macroparásitos del cíclido de Midas, de otros cíclidos y de peces de otras familias coexistentes, en cinco lagos cráter y los dos lagos grandes de Nicaragua.

Los análisis morfológicos y moleculares revelaron una fauna de macroparásitos compuesta por 42 taxones, 37 de los cuales infectan a cíclidos. La mayoría corresponde a especies ya descritas en cíclidos de Mesoamérica. La diversidad de parásitos incluye especies de platelmintos, nematodos, copépodos, branquiuros, hirudineos y ácaros oribátidos. Entre la fauna parasitaria se incluyen tres especies invasoras que se reportan por primera vez. Además, describimos dos especies nuevas. Los lagos grandes tuvieron una diversidad de parásitos mayor que los lagos cráter más pequeños y aislados. El cíclido de

Midas estuvo infectado por 22 taxones de parásitos, 18 compartidos con otros cíclidos. Encontramos una fauna núcleo de parásitos en el cíclido de Midas, que comprende parásitos filogenéticamente distantes, con historias de vida contrastantes y distintos grados de especificidad hospedatoria. En conjunto, la diversidad de parásitos de los peces en sistemas de agua dulce de Nicaragua mostró una diversidad notable, y comprende 101 taxones reportados en 51 de las 114 especies de peces de agua dulce conocidas para el país.

Encontramos que las comunidades de parásitos son más similares en las poblaciones simpátricas que en las alopátricas del cíclido de Midas, formando ensambles únicos dentro de cada lago, con estabilidad temporal. Observamos patrones de abundancias parasitarias paralelas entre ecomorfotipos equivalentes a lo largo de las radiaciones. Los paralelismos fueron encontrados cuando se consideraron los perfiles de infección por taxón de parásito individual, cuyas estrategias de transmisión reflejan los paralelismos tróficos e historias de vida de sus hospederos. Las interacciones entre especies en cada una de las comunidades de parásitos mostraron coocurrencias no aleatorias, aunque no paralelas entre los ecomorfotipos equivalentes de sus hospederos.

El estudio que se presenta en esta tesis sugiere que las interacciones hospedero-parásito pudieron haber tenido un papel en las radiaciones adaptativas del cíclido de Midas desde estadios incipientes de la especiación. Asimismo, nuestro estudio resalta que los cíclidos de Midas y sus parásitos representan un modelo potencial para explorar los mecanismos que promueven la diversidad.

ABSTRACT

Hosts and parasites are engaged in complex interactions of constant reciprocal adaptation, imposing strong selective forces to each other capable of altering their evolutionary trajectories. During the colonization of a new environment, hosts may lose parasites and maintain only a subset of their original diversity, generating new parasite assemblages. The exposure of hosts to contrasting parasite communities can impose different selective pressures among populations and trigger or accelerate the divergence of their hosts. The Midas cichlid is an excellent model to study the mechanisms driving speciation, both in sympatry and in allopatry, and the evolution of parallelisms. The replicated ecological framework and the temporal variation of the evolution within crater lakes, in addition to the recent or ongoing diversification, provide an overview of the initial conditions that caused divergence, and a glimpse to approach the mechanisms guiding the divergence in a continuum of speciation.

In this study, we investigated the potential role of parasite mediated selection in the adaptive radiations of the Midas cichlids. If populations inhabiting different environments are exposed to different parasite communities, and these are temporally stable, this can fuel diversification. We characterized the parasite communities in the Midas cichlids, other coexisting cichlids, and non-cichlids over three consecutive years, in the two great lakes and five crater lakes in Nicaragua.

The morphological and molecular analyses revealed a macroparasite fauna composed by 42 taxa, 37 of them infecting cichlids. Most of the parasites were already described in other Middle American cichlids. Parasite diversity includes species of platyhelminths, nematodes, copepods, branchiurans, hirudineans and oribatid mites. Among these parasites, three invasive species were reported for the first time. Furthermore, we described two new species. The large lakes had larger parasite diversity than the smaller and more isolated crater lakes. The Midas cichlid was infected by 22 parasite taxa, 18 shared with other cichlids. We found a core parasite fauna in the Midas cichlid composed by eight parasite taxa, which comprise distantly related groups, with contrasting life histories and degrees of host specificity. The overview of the parasite diversity in fishes from the Nicaraguan freshwater systems showed remarkable diversity but still, many

gaps in the research. The parasite fauna comprises 101 taxa in 51 of the 114 fish species currently known in the country.

We found that parasite communities are more similar among sympatric than allopatric Midas cichlid populations, forming unique assemblages within lakes, stable over time. We uncovered signs of abundant parallelisms between equivalent ecomorphotypes across crater lake radiations. The similarities were found when considering the infection profiles by individual parasite taxa, whose transmission strategies reflect the trophic parallelisms and life histories of their hosts. The species interactions within parasite assemblages displayed non-aleatory co-occurrences, although non-parallel in equivalent host ecomorphotypes.

The study presented in this thesis suggests that host-parasite interactions may have played a role on the Midas cichlid adaptive radiations since early stages of divergence. Furthermore, our results highlight the idea that the Midas cichlids and their parasites represent a promising model to explore the mechanisms triggering biodiversity.

Publicaciones originales asociadas a la tesis (publicadas o en preparación)

Los artículos producidos como resultado del proyecto de doctorado se muestran en orden cronológico:

Artículo I

Santacruz A., Morales-Serna F.N., Leal-Cardín M., Barluenga M. & Pérez-Ponce de León G. (2020) *Acusicola margulisae* n. sp. (Copepoda: Ergasilidae) from freshwater fishes in a Nicaraguan crater lake based on morphological and molecular evidence. *Systematic parasitology*, **97**, 165–177.

Artículo II

Santacruz A., Barluenga M. & Pérez-Ponce de León G. (2021) Taxonomic assessment of the genus *Procamallanus* (Nematoda) in Middle American cichlids (Osteichthyes) with molecular data, and the description of a new species from Nicaragua and Costa Rica. *Parasitology Research*, **120**, 1965–1977.

Artículo III

Santacruz A., Barluenga M. & Pérez-Ponce de León G. (2022) Macroparasite diversity in cichlid fish from Nicaraguan lakes: first steps towards understanding host-parasite diversification and speciation in a model system. *Scientific Reports* (*in press*).

Artículo IV

Santacruz A., Barluenga M. & Pérez-Ponce de León G. (2022) Filling the knowledge gap of Middle American freshwater fish parasite biodiversity: Metazoan parasite fauna of Nicaragua. *Journal of Helminthology* (*in press*).

Artículo V

Santacruz A., Pérez-Ponce de León G. & Barluenga M. (2021) Divergent parasite assemblages in young adaptive radiations: parasite-mediated selection in the Midas cichlid? Manuscrito en preparación para *American Naturalist.*

CAPÍTULO 1. Introducción

Las interacciones hospedero-parásito representan una de las asociaciones ecológicas más comunes en la naturaleza, y podría ser responsable de las trayectorias evolutivas de ambos miembros de la interacción (Haldane, 1949). Durante la colonización de un nuevo ambiente, el hospedero puede perder alguno o todos sus parásitos y adquirir nuevos parásitos del nuevo entorno, resultando en nuevos ensambles (Hoberg *et al.*, 2012). Los cambios en estas dinámicas pueden alterar la fuerza de las interacciones (Wolinska *et al.*, 2008; Best *et al.*, 2017), y producir cambios adaptativos rápidos (Eizaguirre *et al.*, 2012). La fuerte presión de selección que los parásitos ejercen sobre sus hospederos puede tener implicación en cambios de comportamiento, o en la selección de hábitat que puede favorecer o prevenir infecciones (Eizaguirre & Lenz, 2010; Mikheev *et al.*, 2013; Demandt *et al.*, 2018; Jolles *et al.*, 2020), o en la elección de pareja, influyendo directa o indirectamente sobre rasgos sexuales (Milinski, 2014). La presión de los parásitos desencadena mecanismos de defensa. En vertebrados, existen genes altamente polimórficos del sistema inmune, particularmente el complejo mayor de histocompatibilidad (MHC) que cambian rápidamente como respuesta a los parásitos locales (Eizaguirre *et al.*, 2012).

La especiación en ausencia de barreras geográficas representa un reto conceptual sobre cómo las especies divergen manteniendo algunos niveles de flujo genético. En este contexto, el efecto de los parásitos podrían ser un factor que facilite la divergencia en simpatría (Eizaguirre & Lenz, 2010). En la especiación en simpatría, el papel de la ecología en la formación de especies (especiación ecológica) se ha postulado como el mecanismo principal, asociado por ejemplo a una diferenciación de nicho entre las poblaciones (Losos *et al.*, 1998). A medida que los estudios se vuelven más rigurosos sobre la trayectoria evolutiva de las poblaciones, más y más ejemplos en la naturaleza muestran que lo que se pensaba como especiación en simpatría, estuvo sujeto a eventos de colonizaciones secundarias, eventos de alopatría pasados o hibridación (Martin *et al.*, 2015; Richards *et al.*, 2018b). Uno de los ejemplos más sólidos de especiación en simpatría es el de el cíclido de Midas (Barluenga *et al.*, 2006), peces que evolucionaron en ambientes pequeños y aislados, donde es posible poner a prueba el cumplimiento de los supuestos de simpatría (Coyne & Orr, 2004; Coyne, 2011).

El cíclido de Midas es un grupo de peces neotropicales de agua dulce que habita una serie de lagos cráter en Nicaragua. Las poblaciones de los lagos cráter derivan de dos lagos grandes de origen tectónico, estableciendo un escenario evolutivo análogo al modelo continente-isla (Barluenga & Meyer, 2010; Kautt *et al.*, 2020). La heterogeneidad temporal de la colonización, y la diferenciación biótica y abiótica dentro de los lagos cráter, forma un mosaico geográfico de posibilidades evolutivas, representando réplicas en las que se pueden estudiar las dinámicas de la interacción hospedero-parásito. En cada uno de los lagos, el cíclido de Midas ha experimentado radiaciones adaptativas de forma reciente, dando lugar a grupos de especies con nichos bien diferenciados (Elmer *et al.*, 2010a). Los nichos análogos en distintos lagos se traducen en peces con ecomorfotipos paralelos en varios ejes de diferenciación, con peces con cuerpos elongados típicos de aguas abiertas de varios lagos, y peces de cuerpos profundos que habitan las zonas bénticas superficiales o profundas (Elmer *et al.*, 2014; Kusche *et al.*, 2014; Kautt *et al.*, 2016), labios gruesos ligados a una alimentación entre las rocas (Machado-Schiaffino *et al.*, 2014), o formas en las mandíbulas faríngeas que varían dependiendo de una dieta blanda, dura o mixta (Muschick *et al.*, 2011). Además, existen variaciones en la coloración que pueden ocurrir en todos los ecomorfotipos (Barlow, 1983).

Los nichos contrastantes imponen una exposición diferencial a los parásitos (MacColl, 2009). Las rutas de transmisión para la adquisición de un parásito están mediadas en parte por la historia de vida del hospedero (Hayward *et al.*, 2017). Es decir, el tipo de hábitat determina las oportunidades de adquirir cierto parásito, formando ensambles de parásitos más diferenciados en hospederos con nichos no superpuestos (Meyer *et al.*, 2019). Las comunidades de parásitos contrastantes representan adaptaciones locales de una población de hospederos, las cuales, ligadas a la selección natural y/o sexual, podrían potenciar, mantener o acelerar las diferencias entre las poblaciones (Page, 2003; Hund *et al.*, 2020), limitando el flujo genético entre éstas. Esto implica que los parásitos podrían representar una fuerza que forma parte de la serie de mecanismos que permiten la especiación en simpatría. Karvonen & Seehausen (2012) propusieron tres pre-requisitos para sustentar la hipótesis de especiación mediada por parásitos: 1) que los ensambles de parásitos sean distintos entre las poblaciones, 2) que la dirección de las diferencias mantenga una estabilidad temporal y 3) que el efecto de los parásitos sobre la adecuación (*fitness*) del hospedero sea superior al efecto de otros factores.

1.1 Hipótesis

Las poblaciones del cíclido de Midas tienen un ensamble contrastante de parásitos que podría estar contribuyendo a la divergencia de sus hospederos.

Predicciones

-Las comunidades de parásitos van a diferir en los diferentes lagos.

-Las comunidades de parásitos van a diferir en hospederos con diferentes preferencias tróficas y de hábitat.

-Las poblaciones de peces con diferente coloración tendrán diferentes comunidades de parásitos si tienen hábitos vitales distintos.

1.2 Objetivos

Investigar la composición e historia evolutiva de las especies de parásitos del cíclido de Midas en los lagos de Nicaragua para entender si éstos tienen el potencial de favorecer cambios evolutivos que se puedan relacionar con los procesos de especiación repetida y en paralelo de sus hospederos. Para este propósito, planteamos las siguientes preguntas:

1) ¿Qué parásitos infectan a los peces de los lagos de Nicaragua?
2) ¿Cómo se distribuye espacialmente la diversidad de parásitos en los lagos?
3) ¿Existen diferencias en las comunidades de parásitos entre las especies del cíclido de Midas?
4) ¿Existe estabilidad temporal en las comunidades de parásitos?
5) ¿Qué factores estructuran las comunidades de parásitos?
6) ¿Qué interacciones se establecen entre los diferentes parásitos de una comunidad?

CAPÍTULO 2. Antecedentes

2.1 La especiación simpátrica

Islas remotas o pequeños lagos cráter representan los escenarios ideales para probar la especiación sin aislamiento geográfico y en presencia de flujo genético, es decir, la especiación en simpatría (Coyne, 2011). Los cuatro supuestos a cumplir para la especiación en simpatría de acuerdo con Coyne & Orr (2004) son: i) distribución simpátrica actual, ii) aislamiento reproductivo, iii) especies hermanas filogenéticamente y iv) no aislamiento geográfico previo. Para que la especiación en ausencia de barreras geográficas ocurra, la selección natural debe operar en direcciones opuestas en distintas poblaciones (Nosil *et al.*, 2021), y debe ser superior al efecto homogeneizador del flujo genético. Si las poblaciones se adaptan a distintos nichos (diferente profundidad o distintos parásitos), puede operar un '*carácter mágico',* es decir, un rasgo con carácter pleiotrópico, que afecta de forma simultanea dos rasgos (Gavrilets, 2018). En este contexto, por ejemplo, 'te reproduces donde comes', promoviendo una divergencia simpátrica. Los eventos de simpatría son raros, pero quizá ocurren en la naturaleza con más frecuencia de lo que pensamos, aunque son difíciles de discernir en presencia de barreras geográficas actuales o divergencias ancestrales. En ambientes pequeños como islas o lagos volcánicos donde es improbable la presencia de barreras geográficas para aislar a las poblaciones, se han descrito los ejemplos más claros de especiación en simpatría: cíclidos en los lagos de Camerún (Richards *et al.*, 2018a) y cíclidos de Midas en lagos cráter de Centroamérica (Barluenga & Meyer, 2004; Barluenga *et al.*, 2006; Kautt *et al.*, 2020). En la era genómica muchos casos de simpatría se han descartado al revelar múltiples colonizaciones e introgresión genética entre poblaciones que han evolucionado en alopatría, de modo que vertientes nuevas evaluan el efecto de la introgresión durante la divergencia (Richards *et al.*, 2018b).

2.2 La especiación mediada por parásitos

Distintos mecanismos no mutuamente excluyentes son capaces de explicar el efecto de los parásitos sobre sus hospederos. Los parásitos pueden acelerar la divergencia como respuesta a una mayor diversidad de sus especies (Betts *et al.*, 2018), incrementando la separación de las trayectorias evolutivas entre las poblaciones alopátricas de los hospederos (Buckling & Rainey, 2002). Contrariamente, también podrían limitar la diferenciación y prevenir la especiación simpátrica, al modular la densidad de hospederos y disminuir la competencia por recursos (Buckling & Rainey, 2002). En el contexto de la especialización ecológica, el principal argumento son diferencias en las comunidades de parásitos, las cuales, pueden mediar adaptaciones contrastantes del sistema inmune (Fig. 1) (Eizaguirre *et al.*, 2011), promover una elección diferencial de hábitat en presencia del parásito en ciertos ambientes (Poulin & Fitzgerald, 1989; Stutz *et al.*, 2015), mediar la dispersión (Baines *et al.*, 2020), inducir cambios conductuales (Demandt *et al.*, 2018), o alterar la elección de pareja (Milinski, 2014). Es decir, los parásitos en conjunto con genes del sistema inmune (MHC) tienen efectos pleiotrópicos sobre la divergencia de sus hospederos (Eizaguirre *et al.*, 2009).

Diferentes hábitats están asociados a distintos ensambles de parásitos (e.g., MacColl, 2009). Las rutas de transmisión para la adquisición de un parásito dependen de la historia de vida de su hospedero (Stutz *et al.*, 2014), de rasgos como las preferencias de alimentación, comportamientos sociales, densidad de la población (Hayward *et al.*, 2017). Es decir, las oportunidades de adquirir cierto tipo de parásito dependen del hábitat. Distintos factores pueden modular la intensidad de la infección, por ejemplo, la edad del hospedero (Izhar *et al.*, 2015) o la carga genética heredada (los parásitos a los que hayan sido expuestos los parentales). Las diferencias en los patrones de infección deben ser consistentes a lo largo del tiempo para que se mantenga la dirección de la selección divergente (Karvonen & Seehausen, 2012). Las diferencias constantes en las cargas parasitarias pueden dar lugar a fenotipos divergentes (*speciation phenotypes*) (Shaw & Mullen, 2011). Incluso la variación espacio-temporal de los parásitos genera distintas presiones de selección sobre los hospederos (Penczykowski *et al.*, 2015).

Existen pocos estudios en vertebrados con evidencia de los parásitos como un agente importante en la selección. En el caso de sistemas naturales, muchos ejemplos se derivan de cíclidos, un grupo con una enorme tendencia a diversificar (Meyer, 2017), que alberga una gran diversidad de parásitos (Vanhove *et al.*, 2016). En este sistema se han encontrado faunas de parásitos contrastantes entre peces bénticos y limnéticos, entre peces de lagos o ríos, y diferencias asociadas a la coloración o gradiente de profundidad de los hospederos (Blais *et al.*, 2007; Raeymaekers *et al.*, 2013; Hablützel *et al.*, 2016; Karvonen *et al.*, 2018; Meyer *et al.*, 2019; Gobbin *et al.*, 2020).

Los ejemplos más icónicos derivan de un modelo de estudio de paralelismos evolutivos en el hemisferio norte, el pez espinoso, *Gasterosteus aculeatus*. Los peces que habitan distintos hábitats difieren en sus parásitos, y en ríos se han encontrado menos parásitos que en lagos (Wegner *et al.*, 2003a), o en peces limnéticos se han encontrado parásitos de hábitats limnéticos y peces bénticos infectados por parásitos de áreas bénticas (Stutz *et al.*, 2014). Las diferentes comunidades de parásitos se correlacionan con un sistema inmune distinto (Wegner *et al.*, 2003b; Hablützel *et al.*, 2016), donde los hospederos tienen mayor resistencia a los parásitos con los que han estado expuestos por más tiempo (El Nagar & Maccoll, 2016; Erin *et al.*, 2019). Las hembras son capaces de detectar mediante odorantes la composición del sistema inmune (Andreou *et al.*, 2017; Gahr *et al.*, 2018), y eligen al macho '*óptimo*' para responder a los parásitos locales (Reusch *et al.*, 2001; Gasparini *et al.*, 2015). Estos estudios han robustecido la idea del sistema inmune (*i.e.* complejo mayor de histocompatibilidad, MHC) como un '*carácter mágico'*, el cual está bajo selección natural divergente (seleccionado por los parásitos), conduce a un emparejamiento selectivo, y tiene efectos ecológicos (Eizaguirre *et al.*, 2009; Andreou *et al.*, 2017).

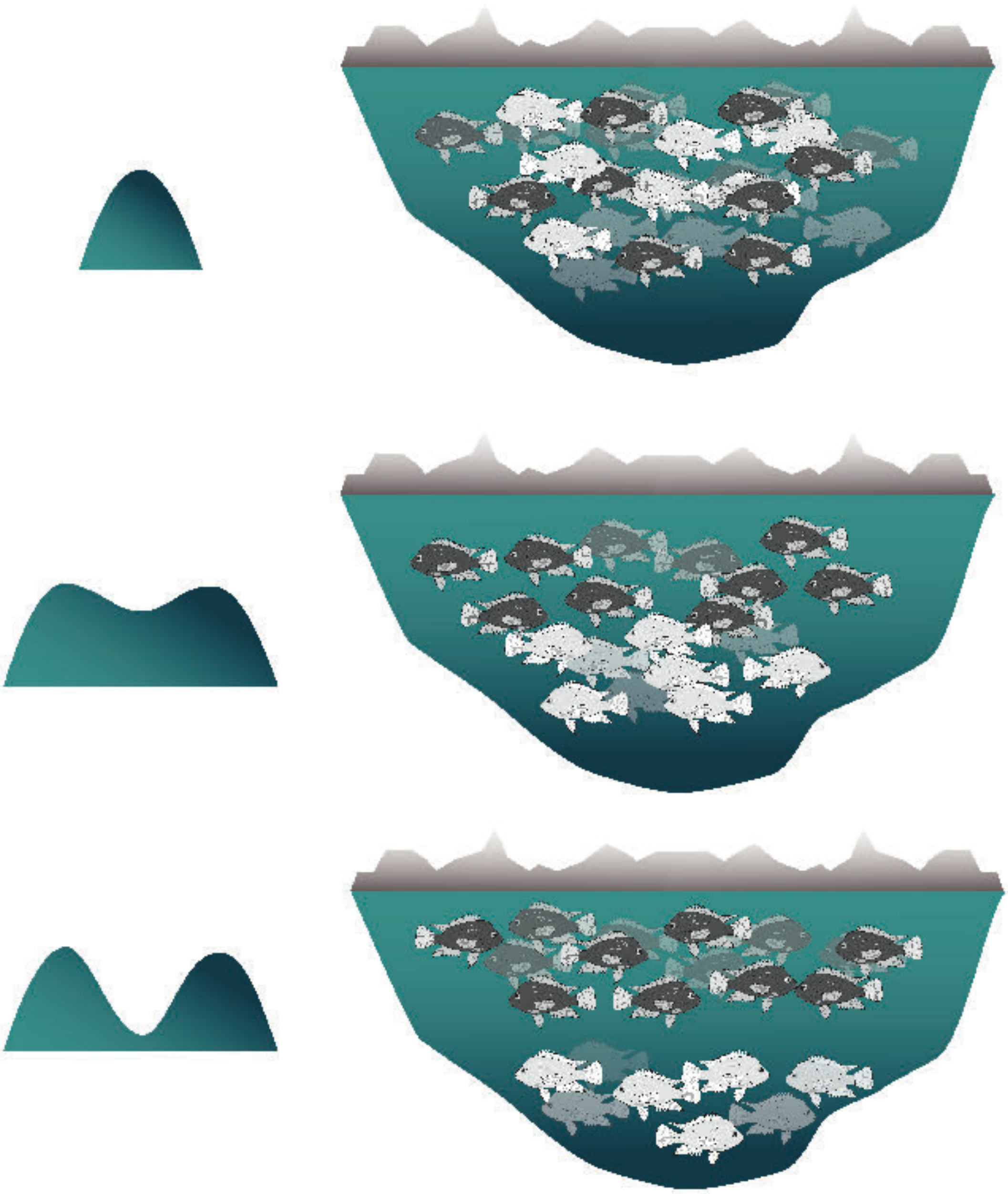

Figura 1. Modelo de especiación en simpatría mediada por parásitos. (A) Poblaciones panmicticas. **(B)** Comienza a haber diferenciación de hábitat y cambian las oportunidades de adquirir ciertos parásitos. **(C)** Si la selección de pareja está ligada al hábitat (‘*mate where you eat*’), esto promueve el aislamiento reproductivo. Imagen adaptada de Eizaguirre & Lenz (2010).

2.3 Colonización hospedero-parásito y la formación de ensambles de parásitos

La colonización de nuevos ambientes implica la dispersión conjunta de hospederos y algunos de sus parásitos, generando nuevos ensambles. En parásitos, la colonización está fuertemente ligada a la interacción con sus hospederos. Cuando el hospedero migra, se mueve entre parches o se extingue, asimismo, el parásito puede replicar los mismos patrones. El parásito puede infectar múltiples linajes de hospederos y replicar por separado la historia de cada linaje. El hospedero generalmente alberga más de una especie de parásito, que también podría compartir la misma historia (Whiteman *et al.*, 2007). Sin embargo, el parásito puede trazar su propia historia evolutiva desligada de su hospedero (Jorge *et al.*, 2017). Tras la colonización puede ocurrir un efecto de síndrome de isla, y en los modelos continente-isla se refleja en una menor riqueza de parásitos conforme aumenta la distancia con el continente (Pérez-Rodríguez *et al.*, 2013). Asimismo, los parásitos pueden experimentar pérdida de diversidad genética, ampliación del nicho ecológico (Nieberding *et al.*, 2006), oscilaciones del rango de hospederos (Jorge *et al.*, 2017) o cambios en la especificidad hospedatoria (Tomé *et al.*, 2018).

Los ensambles nuevos que se pueden formar tras la colonización están mediados por factores estocásticos (Gleason, 1926) y determinísticos (Clements, 1936). Un patrón común son comunidades de parásitos heterogéneas a lo largo del área de distribución de un hospedero (Poulin & Morand, 1999; Poulin & Valtonen, 2002). Las variaciones locales crean ensambles de parásitos anidados conformados por un subgrupo de la diversidad total de parásitos que infecta a una especie de hospedero (Poulin & Valtonen, 2001). La riqueza del ensamble está determinada por rasgos del hospedero o factores abióticos dependientes de la escala (Bolnick *et al.*, 2020). En el caso de las islas, se ha hipotetizado que la riqueza de especies está determinada por su tamaño, distancia del continente y riqueza de especies en el continente (MacArthur & Wilson, 2001; Poulin, 2004).

En la medición de la riqueza de un ensamble de parásitos se emplean comúnmente el valor neto de la riqueza (número de especies) y la homogeneidad de las infecciones, que incluye los índices de diversidad más usados como Shannon-Wiener o Simpson. Sin embargo, nuevas metodologías que consideran la señal filogenética del ensamble plantean una aproximación potencialmente más real de la diversidad. La premisa es que parásitos más distantes filogenéticamente formarán ensambles de parásitos más diversos. Una aproximación metodológica prometedora para considerar la señal filogenética del ensamble y la abundancia relativa de cada especie de parásito es el '*phylogenetic species evenness'* (PSE) (Helmus *et al.*, 2007). Los valores bajos de este índice indican un ensamble con especies de parásitos cercanamente relacionados y con abundancias heterogéneas. El valor máximo del PSE se alcanza cuando los parásitos son igualmente abundantes y la topología de su árbol filogenético tiene forma de estrella.

2.4 El escenario geográfico de la evolución del cíclido de Midas y sus parásitos

Los lagos cráter de Nicaragua son un laboratorio natural para estudiar la evolución. Los lagos cráter como las islas oceánicas, son hábitats geográficamente aislados, ideales para explorar los mecanismos que disparan la biodiversidad. El sistema comprende dos lagos tectónicos conectados intermitentemente, el Lago Nicaragua y el Lago Managua. Adyacentes a los grandes lagos hay una serie de lagos cráter que difieren en tamaño, edad y hábitats (Fig. 2-3). El sistema representa un modelo análogo al modelo continente-isla, con los grandes lagos (‘*los continentes*’) actuando como fuentes que han poblado los lagos cráter (‘*las islas*’) (e.g. Barluenga & Meyer, 2010; Elmer *et al.*, 2012). Los lagos grandes surgieron durante el Pleistoceno, mientras que los lagos cráter se formaron hace apenas unos 24,000 a 1,600 años debido a la actividad volcánica (Kutterolf *et al.*, 2007; Avellán *et al.*, 2014). Cuando la actividad cesó, los volcanes colapsaron, y los cráteres se llenaron de agua y de alguna forma también de vida. Dada la formación reciente de los lagos cráter, albergan poblaciones en estadios tempranos de divergencia. La mayoría de los lagos cráter no están en contacto con otros cuerpos de agua. Los lagos cráter albergan una menor riqueza de especies que los lagos grandes (Waid *et al.*, 1999; Santacruz *et al.*, 2022), con biotas relativamente más simples. Las diferentes edades de los lagos ofrecen un gradiente temporal para estudiar los cambios después de una nueva colonización, por ejemplo, en interacciones hospedero-parásito. Cada lago cráter representa un episodio de expansión geográfica o colonización y aislamiento para el hospedero y parásito, que depende de la persistencia del parásito en su hospedero tras el cambio de ambiente.

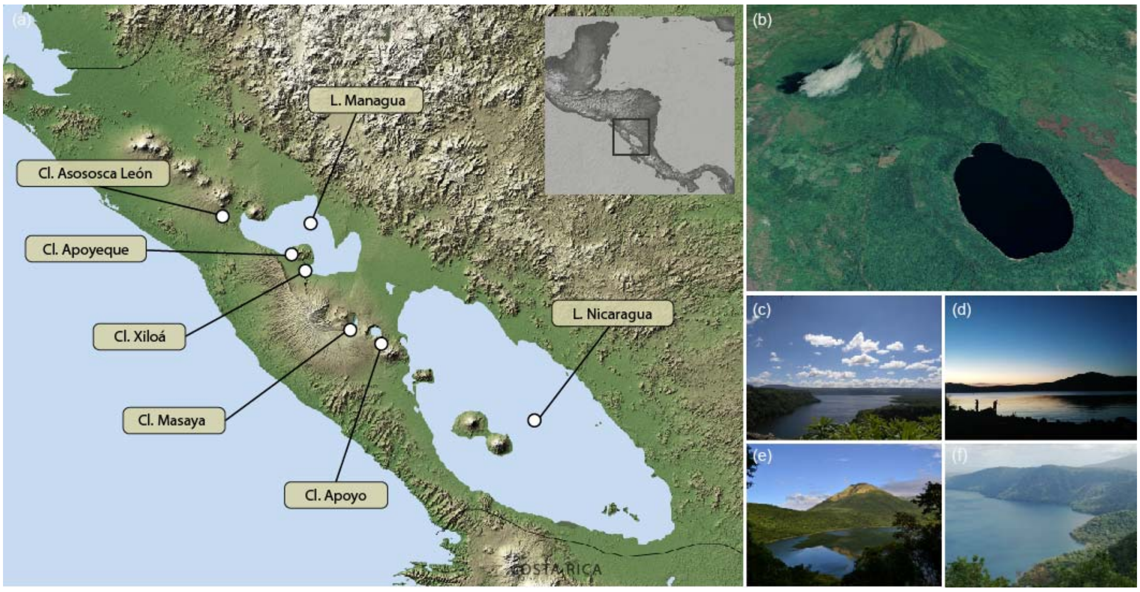

Figura 2. Mapa de los lagos de Nicaragua. (**a**) En el mapa se indica la ubicación de los grandes lagos y lagos cráter de este estudio. (**b**) Vista aérea del lago cráter Asososca León. Fotografías de los lagos cráter: (**c**) Masaya, (**d**) Xiloá, (**e**) Asososca León y (**f**) Apoyo.

2.5 Las radiaciones adaptativas y en paralelo del cíclido de Midas

Los cíclidos han experimentado algunas de las radiaciones adaptativas más impresionantes que se conocen. Tan sólo las explosiones de diversificación ocurridas en los últimos pocos millones de años en los Grandes Lagos del Este de África han dado lugar a cientos de especies: unas 250 especies en el Lago Tanganika, unas 700 especies en la región del Lago Victoria y cerca de 800 en el Lago Malawi (Salzburger, 2018). Una radiación adaptativa más reciente ha tenido lugar en los lagos de Centro América (Barluenga & Meyer, 2004, 2010; Barluenga *et al.*, 2006; Kautt *et al.*, 2020). La gran propensión de los cíclidos para evolucionar está ligada a innovaciones evolutivas, variación genética críptica e hibridación (Meier *et al.*, 2017; Salzburger, 2018), cambios que ocurren a tasas muy rápidas en los linajes más propensos a evolucionar (Seehausen, 2015). Los factores extrínsecos asociados a la rápida diversificación de los cíclidos incluyen la variación del hábitat (Ivory *et al.*, 2016), cambios tróficos y conductuales (York *et al.*, 2015; Burress *et al.*, 2020) y la exposición a distintos parásitos (Raeymaekers *et al.*, 2013; Meyer *et al.*, 2019; Gobbin *et al.*, 2020).

El complejo de especies del cíclido de Midas (*Amphilophus* spp.) comprende cerca de 10 especies que habitan los dos grandes lagos Managua y Nicaragua y varios lagos cráter (Fig. 3). Los cíclidos de Midas comprenden recientes radiaciones adaptativas, algunas apenas en un proceso incipiente de especiación (Kautt *et al.*, 2016; Machado-Schiaffino *et al.*, 2017). Dentro de estos lagos parecen ser el único linaje que ha experimentado radiaciones adaptativas, dando lugar a un ejemplo clásico de libro de texto de especiación en simpatría. Los tempos y modos de diversificación del cíclido de Midas han sido explorados extensivamente (Barluenga & Meyer, 2004; Barluenga *et al.*, 2006; Elmer *et al.*, 2010a). Se estima que el cíclido de Midas colonizó los lagos cráter a partir de poblaciones derivadas de los grandes lagos hace unos 4,700 a 800 años (Fig. 4) (Kautt *et al.*, 2020). La colonización fue seguida por una especialización de nicho y especiación intralacustre (Barluenga *et al.*, 2006; Elmer *et al.*, 2010b). Como resultado, los cíclidos de Midas que habitan nichos equivalentes presentan ejes de diferenciación paralelos (Muschick *et al.*, 2011; Colombo *et al.*, 2013; Elmer *et al.*, 2014).

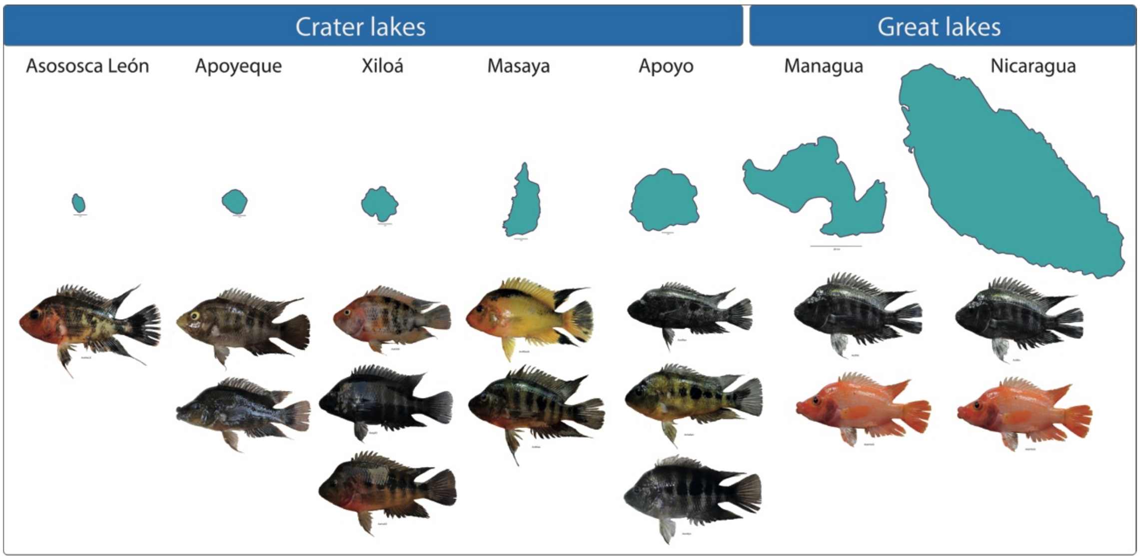

Figura 3. Las radiaciones adaptativas del cíclido de Midas. Representación esquemática de los morfotipos principales en los lagos de Nicaragua.

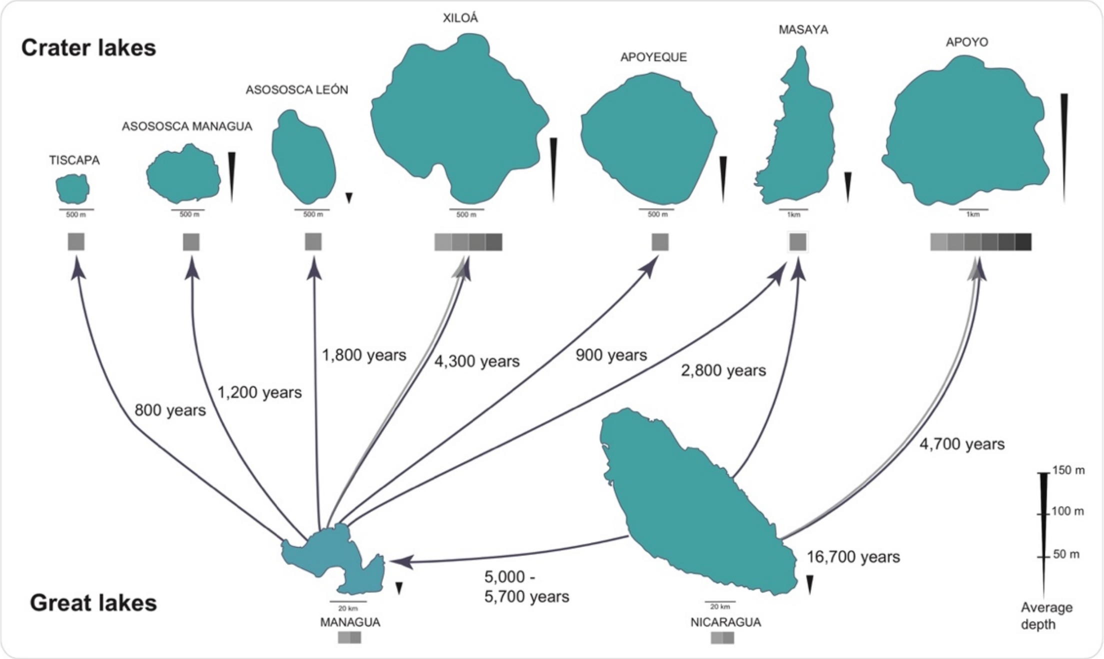

Figura 4. Patrón de colonización de los lagos. Los tiempos estimados y rutas de colonización se basan en el estudio Kautt *et al.* (2020). El recuadro debajo de cada lago indica el número de especies de cíclidos de Midas descritas para dicho lago.

2.5.1 Paralelismos

Los principales ejes de diferenciación en el cíclido de Midas son la forma del cuerpo, la mandíbula faríngea y el grosor de los labios, caracteres donde parece residir la mayor variación fenotípica asociada a las adaptaciones a nuevos ambientes (Fig. 5). Estos fenotipos se replican a lo largo de los lagos en hábitats similares dando lugar a fenotipos paralelos (ecomorfos o ecomorfotipos), al igual que sucede en cíclidos de los Grandes Lagos del Este de África (Salzburger, 2018). El paralelismo en la forma del cuerpo es evidente en las radiaciones de los lagos más profundos, los lagos cráter Xiloá y Apoyo (Elmer *et al.*, 2014). En estos lagos existe una extraordinaria repetición de una forma limnética y múltiples formas bénticas adaptadas a profundidades distintas. Las formas limnéticas con cuerpos elongados habitan las zonas de aguas abiertas, mientras que peces con cuerpos más altos habitan zonas bénticas, superficiales o profundas, y maniobran entre las rocas y la vegetación (Elmer *et al.*, 2014; Kusche *et al.*, 2014; Kautt *et al.*, 2016).

El fenotipo de labios gruesos (*Amphilophus labiatus*) habita los grandes lagos Managua y Nicaragua, aunque formas similares se encuentran en baja frecuencia en los lagos cráter Apoyeque, Xiloá y Masaya (Barlow & Munsey, 1976; Manousaki *et al.*, 2013). Los peces con labios gruesos o delgados ocupan nichos distintos en los lagos; los peces con labios gruesos están ligados a las rocas (Machado-Schiaffino *et al.*, 2014). La mandíbula faríngea es considerada una novedad evolutiva en los cíclidos, otorgando una mayor flexibilidad en la dieta (Meyer, 2017). En los cíclidos de Midas las formas de esta estructura varían dependiendo de su dieta, con dientes molariformes, papiliformes o formas mixtas (Muschick *et al.*, 2011).

Una de las características más llamativas en los cíclidos de Midas es su policromatismo, con fenotipos que van del amarillo al rojo llamados *gold*. Este fenotipo tiene bajas frecuencias en los lagos, siendo más común en aguas turbias. Por ejemplo, se encuentra totalmente ausente en las aguas cristalinas del lago cráter Apoyo. Los peces nacen con una coloración oscura -llamada *dark*-, y el cambio en algunos de los peces comienza entre los tres a doce meses (Barlow, 1976; Sowersby *et al.*, 2015). El cambio tardío de la coloración implica que algunos peces *gold* podrían tener eventos reproductivos con su coloración *dark*.

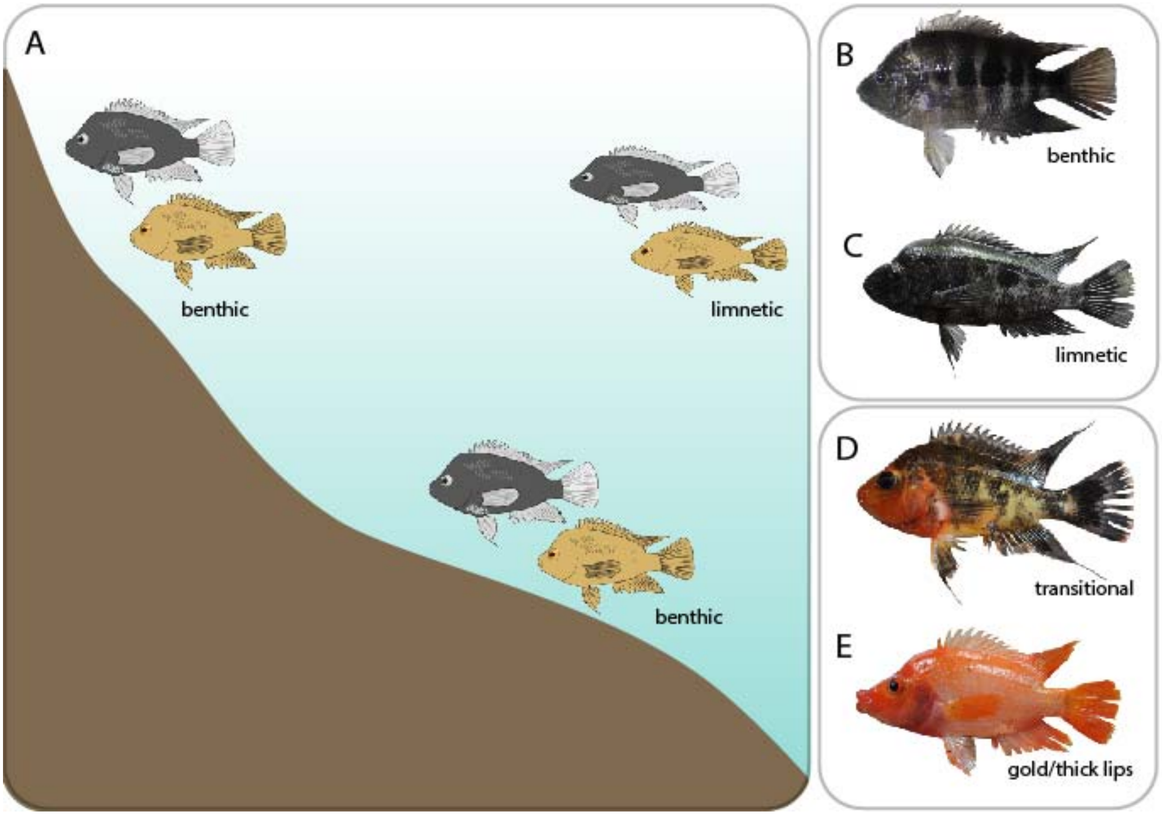

Figura 5. Paralelismos en ejes de diferenciación y coloración. (**A**) Las poblaciones del cíclido de Midas habitan nichos diferenciados dentro de los lagos, como zonas bénticas superficiales o profundas y zonas limnéticas. Los paralelismos más evidentes asociados a su especialización

de nicho se dan en la forma del cuerpo. (**B**) Formas bénticas con cuerpos profundos habitan la zona litoral. (**C**) Formas limnéticas con cuerpos elongados tienden a ocurrir en aguas abiertas. Los cíclidos de Midas nacen con una coloración *dark*, después, sólo algunos individuos transicionan a una coloración *gold.* Ambas coloraciones *gold* y *dark* pueden tener fenotipos de labios delgados (*thin lips*) o gruesos (*thick lips*) (**D**-**E**) y formas del cuerpo bénticas o limnéticas.

2.6 Estudios previos de los parásitos de peces en los lagos de Nicaragua

En el Neotrópico se ha hecho un enorme esfuerzo por documentar la diversidad de parásitos de peces de agua dulce, aunque todavía hay un muchas lagunas de conocimiento en Centro América (Choudhury *et al.*, 2016), incluyendo los lagos de Nicaragua. La contribución más importante fue hecha por Watson (1976), que describió algunos trematodos en peces del Lago Nicaragua, y algunas especies de monogeneos y cestodos de tiburones del mismo lago (Watson & Thorson, 1976). Posteriormente, hubo otros estudios aislados en las vertientes del Atlántico y del Pacífico (Aguirre-Macedo *et al.*, 2001b; a; Vidal-Martínez *et al.*, 2001; Mendoza-Franco *et al.*, 2003; Andrade-Gómez *et al.*, 2017; López-Jiménez *et al.*, 2018). Al inicio de este trabajo se habían reportado 16 especies en el sistema de lagos en siete familias de peces, la mayoría en cíclidos. Nueve especies se reportaron en los grandes lagos Managua y Nicaragua (Watson, 1976; de Chambrier & Vaucher, 1984). Hasta el inicio de nuestro trabajo sólo existía un estudio en los lagos cráter, donde González-Solís & Jiménez-García (2006) reportaron seis especies de nematodos en cíclidos de los lagos cráter Xiloá y Apoyo.

CAPÍTULO 3. El descubrimiento de nuevas especies de parásitos

Como parte de la caracterización de la fauna de parásitos con la mayor resolución taxonómica posible, empleamos aproximaciones morfológicas y moleculares que nos han permitido identificar dos especies nuevas, y aún quedan más por describir. La primera especie corresponde a un copépodo descrito de uno de los lagos cráter más significativos de nuestro estudio, Asososca León. *Acusicola margulisae* forma parte de la diversidad única que habita este lago, infectando a casi todas las especies de peces en altas intensidades (Artículo I). La segunda descripción corresponde al nematodo *Procamallanus barlowi,* una especie altamente específica de cíclidos (Artículo II). Esta especie está ampliamente distribuida en casi todos los lagos, y extiende su distribución hasta ríos en Costa Rica. La relación hermana con otra especie de cíclidos refleja la evolución conjunta de este grupo de parásitos y sus hospederos.

Este capítulo incorpora los manuscritos de cada una de las especies nuevas que han sido o están en el proceso de una descripción formal.

3.1 El copépodo *Acusicula margulisae*

Artículo I

Santacruz A, Morales-Serna N, Leal-Cardín M, Barluenga M & Pérez-Ponce de León G. (2019) *Acusicola margulisae* n. sp., a parasitic copepod (Copepoda: Ergasilidae) from freshwater fish in a Nicaraguan crater lake, described with morphology and molecular data. *Systematic Parasitology*, **97**, 165–177.

3.2 El nematodo *Procamallanus barlowi*

Artículo II

Santacruz A, Barluenga M & Pérez-Ponce de León G. (2021) Taxonomic assessment of the genus *Procamallanus* (Nematoda) in Middle American cichlids (Osteichthyes) with molecular data, and the description of a new species from Nicaragua and Costa Rica. *Parasitology Research*, **120**, 1965–1977.

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Acusicola margulisae n. sp. (Copepoda: Ergasilidae) from freshwater fishes in a Nicaraguan crater lake based on morphological and molecular evidence

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Abstract The ergasilid copepod *Acusicola margulisae* n. sp. is described based on material from three species of cichlid, *Amphilophus citrinellus* (Günther), *Parachromis managuensis* (Günther), and *Oreochromis* sp., and from the poecilid *Poecilia mexicana* (Steindachner), in the crater Lake Asososca León, Nicaragua. This constitutes the 15th species described in the genus *Acusicola* Cressey, 1970. The new species differs from all its congeners by the relatively longer first endopodal segment of leg 1, and the size and number of setae on second endopodal segment of leg 1. We provide the first gene sequence for a species of *Acusicola.* To examine the intraspecific genetic variation of the new species collected from different host species, sequences of the mitochondrial barcode region *cox*1 were generated. In addition, partial regions of the 18S and 28S ribosomal RNA genes were sequenced and used to infer the phylogenetic relationships of the genus *Acusicola* within the family Ergasilidae Burmeister, 1835. The phylogenetic trees yielded the isolates of *Acusicola margulisae* n. sp. as a reciprocally monophyletic lineage, and as the sister taxa of five genera of ergasilid copepods. The genus *Ergasilus* von Nordmann, 1832 was recovered as a paraphyletic group. These analyses indicate that phylogenetic relationships are not yet well resolved and more representative species and genera of the family are required to provide a robust classification of this highly diverse group of copepods.

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Introduction

Copepods are the most abundant group of crustaceans, containing mainly free-living organisms, and a few parasitic lineages that infect predominantly fishes (Klompmaker & Boxshall, 2015). Within the entirely parasitic family Ergasilidae only females are adapted to a parasitic life-style. All developmental stages in the life-cycle in both sexes are free-living, and only after fertilization the female infects its host (Boxshall & Defaye, 2008). Species of *Acusicola* Cressey & Collette, 1970 are widely distributed since most of them parasitise coastal euryhaline fishes (da Motta et al., 1995). Some of the species of *Acusicola* have been found in the USA and Central America (Cressey & Collette, 1970; El-Rashidy & Boxshall, 1999), but the largest species richness of the genus is found in the River Amazon basin in South America (Luque & Tavares, 2007; Luque et al., 2013). The genus *Acusicola* includes mainly parasitic species that are considered among the most pathogenic copepods (Kearn, 2005). Exceptionally, few species are free-living, inhabiting dwelling freshwater, brackish and marine environments (Araujo & Boxshall, 2001). The genus contains 14 species, differentiated morphologically by leg setation patterns (Araujo & Boxshall, 2001).

The Pacific coast of Nicaragua holds the largest freshwater lakes in Central America. The two large lakes, Managua and Nicaragua, originated due to tectonic activity less than 1 Mya (Bussing, 1976). This region is also relevant because of the existence of several crater lakes of volcanic origin, formed within the last few thousand years (Waid et al., 1999; Barluenga & Meyer 2004). The crater lakes were seeded by waves of colonisation from populations in the large lakes, followed by rapid diversification and sympatric speciation. Crater lakes are ideal model systems for studying very recent speciation events associated with isolation and local adaptation. The Crater lake Asososca León is one of these small and isolated lakes, which attracted special interest due to its degree of isolation and relatively impoverished fauna compared to surrounding lakes. It has an estimated age of a few thousand years (Siebert & Simkin, 2002; Elmer et al., 2010), and its fish fauna is potentially derived from the close-by larger Lake Managua, although the time of colonisation of Lake Asososca Leon is still under debate (see Barluenga & Meyer, 2010). The fish fauna of this lake includes two cichlid species, the Midas cichlid *Amphilophus citrinellus* (Günther) and the jaguar guapote *Parachromis managuensis* (Günther), also present in the surrounding lakes, an introduced cichlid, the African tilapia, *Oreochromis* sp. (Günther), and one poecilid, *Poecilia mexicana* (Steindachner) (see Waid et al., 1999; McCrary et al., 2007; Barluenga & Meyer, 2010).

During a survey of the local freshwater fish parasite fauna of the Lake Asososca León in Nicaragua, we collected ectoparasitic copepods from the gills of both native and introduced fish species. Some of these copepod individuals were found to represent an undescribed species of *Acusicola*. Here, we describe the new species based on morphological and molecular data. In addition, molecular data are used to explore the phylogenetic position of the genus *Acusicola* within the family Ergasilidae Burmeister, 1835. We report the first sequence data for a species of this ergasilid genus.

Materials and methods

Specimen collection

During two fieldwork expeditions at the end of the wet season (November-December 2017, 2018) 75 fish were captured in the crater lake Asososca León, Nicaragua: 48 Midas cichlids (*A. citrinellus*); 17 jaguar guapotes (*P. managuensis*); 6 tilapias (*Oreochromis* sp.); and 4 guppies (*Poecilia mexicana*). Fishes were euthanised with an overdose of tricaine methane sulfonate. The gills were then removed and examined under a stereomicroscope to isolate the parasites. Ectoparasites were preserved in individual vials with 100% ethanol for further morphological and molecular analysis.

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Morphological analysis

For morphological characterisation, some specimens were mounted on separate slides, cleared in lactic acid and then examined under an Olympus SZ61 stereomicroscope, and under a Leica DMLB compound microscope. A subset of the specimens was dissected. Drawings were made with the aid of a drawing tube attached to the compound microscope at magnifications of 400× and 1000×. Drawings were then scanned, redrawn using Inkscape 0.91 software, and assembled into figure plates using Gimp 2.8 software. Measurements were taken using an ocular micrometer and are given in micrometres, as the range, followed by the mean in parentheses. For scanning electron microscopy (SEM), some specimens were dehydrated in a series of ethanol and then subjected to critical-point drying with carbon dioxide, sputter-coated with gold, and then examined with a SEM Hitachi Stereoscan Model SU1510 (Hitachi Ltd, Tokyo, Japan). Copepod body and appendage terminology follows El-Rashidy & Boxhall (1999) and Araujo & Boxshall (2001).

Molecular data generation and phylogenetic analyses

DNA was isolated using DNAzol Reagent (Molecular Research Center, Cincinnati, OH, USA) or Speedtools tissue DNA extraction kit (Biotools, Madrid, Spain) according to the manufacturers̓ instructions. The barcode region of the cytochrome *c* oxidase subunit 1 (*cox*1) gene was amplified using the forward primer 507F (5′-AGT TCT AAT CAT AAR GAT ATY GG-3′; Nadler et al., 2006) and the reverse primer HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′; Folmer et al., 1994). The ribosomal genes 28S and 18S were amplified with the primers designed by Song et al. (2008): 28S rDNA (28SF, 5′-ACA ACT GTG ATG CCC TTA G-3′ and 28SR, 5′-TGG TCC GTG TTT CAA GAC G-3′); 18S rDNA (18SF, 5′-AAG GTG TGM CCT ATC AAC T-3′ and 18SR, 5′-TTA CTT CCT CTA AAC GCT C-3′). The amplification was performed with the following conditions: 94°C for 2 min; 30 cycles of 94°C for 1 min, annealing temperature of 48°C (for *cox*1) or 54°C (for 18S and 28S rDNA), and 72°C for 2 min, with a final extension step at 72 °C for 7 min. The PCR products were purified using ExoSAP-IT (Thermo Scientific, CA, USA) and sequenced in both directions with the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, TX, USA). Sequencing was carried out at the Laboratorio de Secuenciación Genómica de la Biodiversidad y de la Salud (Biology Institute, UNAM, Mexico) or at Macrogen sequencing service (Macrogen Inc., Madrid, Spain). Forward and reverse sequences were assembled using Geneious v7 (Kearse et al., 2012). An alignment was constructed for each molecular marker by adding all sequences available on GenBank (Supplementary Table S1). Each dataset was aligned using Clustal Omega web service (Sievers et al., 2011) and verified in Mesquite v3.10 (Maddison & Maddison, 2016). The model of sequence evolution for each matrix was implemented in the ATGC bioinformatics platform using the Smart Model Selection (SMS) (Lefort et al., 2017), and the AIC criterion of selection. The optimal model of molecular evolution was TN93+G+I for 18S rDNA and GTR+G for 28S rDNA. The mitochondrial data was not used in a phylogenetic reconstruction given the low representation of related homologous sequences in the databases for this molecular marker.

To reconstruct the phylogenetic history of the group, two phylogenetic approaches were used, Maximum Likelihood (ML) and Bayesian inference (BI). The ML analysis were carried out in PhyML 3.0 (Guindon et al., 2010) and nodal support for the tree was assessed thorough bootstrap analysis with 1,000 replicates. The BI analysis was run in MrBayes (Huelsenbeck & Ronquist, 2001) using the CIPRES platform (Miller et al., 2010); the analysis included two simultaneous runs of Markov chain Monte Carlo for 10 million generations, sampling every 500 generations, with a heating parameter value of 0.2 and a "burn-in" of 25%. A 50% majority-rule consensus tree representing the posterior probability distribution of clades was generated. The trees were visualised in FigTree v1.4.4 (Rambaut, 2012). Outgroup species were selected following Song et al. (2008). Based on the rDNA data, the uncorrected p-distance was calculated for comparison among members of the family Ergasilidae, while the mitochondrial dataset was used to assess the levels of intraspecific genetic variation among isolates from different host species. The estimations were performed using the software MEGA7 (Kumar et al., 2016), with a bootstrap procedure based on 10,000 replicates.

Order Cyclopoida Burmeister, 1834
Family Ergasilidae Burmeister, 1835
Genus *Acusicola* Cressey, 1970

Acusicola margulisae n. sp.

Type-host: *Amphilophus citrinellus* (Günther) (Perciformes: Cichlidae), Midas cichlid.
Other hosts: *Parachromis managuensis* (Günther), *Oreochromis* sp. (both Cichlidae) and *Poecilia mexicana* (Steindachner) (Poeciliidae).
Type-locality: Asososca León crater lake (12°25′57.191″N, 86°39′41.687″W), Nicaragua.
Type-material: Colección de Parásitos de Peces del Noroeste del Pacífico at CIAD-Mazatlán, Sinaloa, Mexico (CPPNP): holotype female ex *A. citrinellus* (CPPNP 1375); 6 paratype females ex *P. managuensis* (CPPNP 1376); and 17 paratype females from *Oreochromis* sp. (CPPNP 1377 and 1378). Colección Nacional de Crustáceos, Universidad Nacional Autónoma de México (CNCR): CNCR 35552 (ex *Poecilia* sp.); CNCR 35553 (ex *Oreochromis* sp.); and CNCR 35554 (ex *Amphilophus citrinellus*).
Site on host: Gills.
Representative DNA sequences: MN852694-MN852696 (18S); MN852849-MN852851 (28S); MN854838-MN854870 (*cox*1).
ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Acusicola margulisae* n. sp. is urn:lsid:zoobank.org:act:F7CC2485-5336-484E-8550-B3746C606C88
Etymology: The name of the species is in honour of the late Lynn Margulis for her contributions in the field of evolutionary biology.

Description (Figs. 1–5)

Adult female. [Based on 10 specimens.] Body slender, cyclopiform (Figs. 1A, B, 4A, B, D). Body length 1,000–1,297 (1,172) from anterior margin of prosome to posterior margin of caudal rami. Prosoma consisting of oblong cephalosome and 4 pedigerous somites gradually tapering posteriorly. Dorsal surface of cephalosome with nauplius eye located near frontal margin, inverted T-shape marking, and sensillae. Area between cephalosome and first pedigerous somite depressed, with posterior margin of cephalosome distinct on lateral view (Figs. 1A, 4A), but indistinct in dorsal view (Fig. 1B). Urosome comprising short fifth pedigerous somite, ventrally and laterally expanded genital double-somite and 3 free abdominal somites. Genital double-somite with patch of tiny spinules on medio-ventral surface and row of spinules along postero-ventral margin (Fig. 1C). Abdominal somites decreasing gradually in size from anterior to posterior, each bearing row of spinules on postero-ventral margin. Caudal ramus about 1.25 times as long as wide (Figs. 1C, 4C), furnished with small patch of tiny spinules on anteroventral surface and 4 caudal setae; innermost seta VI (Huys & Boxshall, 1991) longest.

Antennule (Figs. 1D, 5A, B) 5-segmented. First segment longest. Second to fifth segments gradually tapering distally. Setal formula (s, setae; ae, aesthetascs): 12s: 6s: 4s: 2s+ae: 6s+ae. Antenna (Figs. 2A, 5C–E) 4-segmented, comprising short coxobasis, 3-segmented endopod and terminal claw; first endopodal segment longest, about 6 times as long as wide, with transverse striation in distal part and minute setules along both outer and inner margins; second endopodal segment (Fig. 2B) with basal outer process, medial constriction, and forming 2 inner lobes; third endopodal segment smallest (arrowed in Fig. 2B); terminal claw short, curved and with fossa on inner margin near tip. Mandible consisting of 3 blades each with sharp teeth (Fig. 2C). Maxillule bearing 1 short and 2 long setae (Fig. 2C). Maxilla comprising large, unarmed syncoxa with 1 pore and basis, with dense array of curved spinules distally (Fig. 2C). Maxilliped absent.

Swimming legs 1 to 4 (Figs. 2D, 3A, B) biramous. Spinulate wide intercoxal sclerites present between

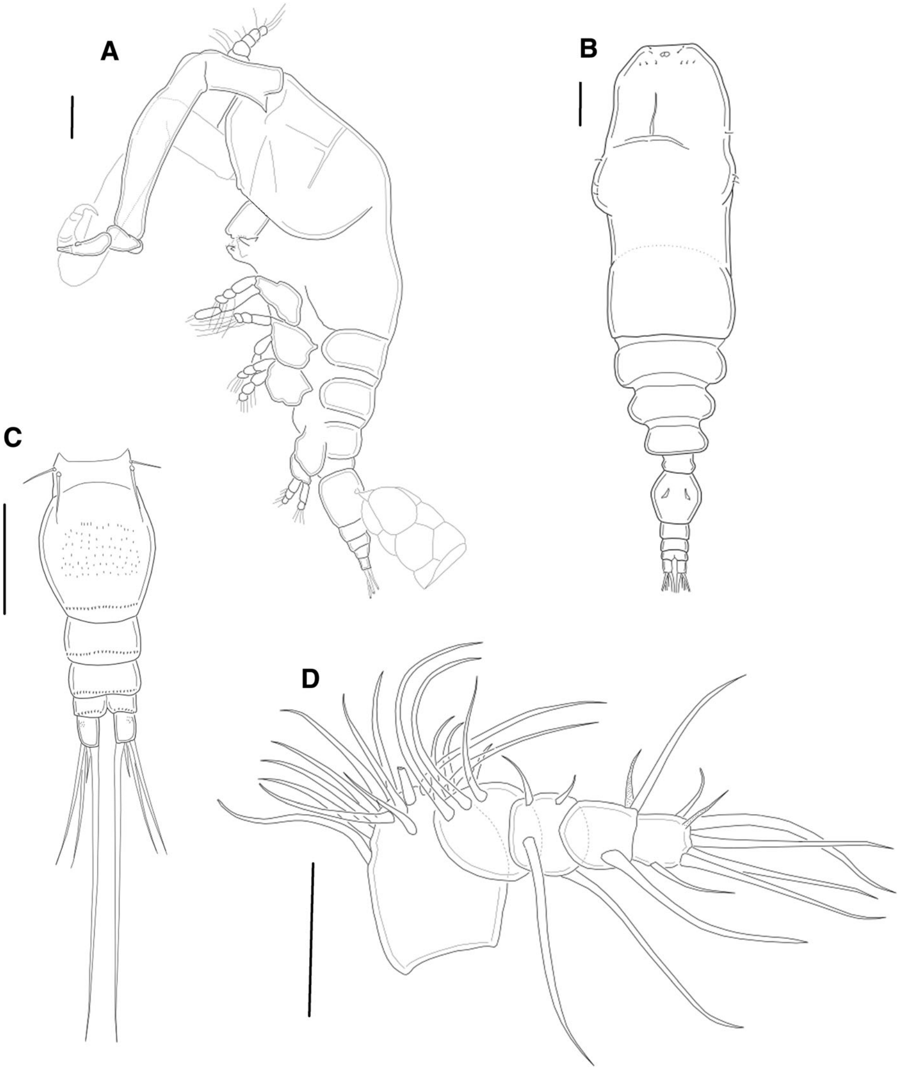

Fig. 1 *Acusicola margulisae* n. sp., holotype female. A, Habitus, lateral view; B, Habitus, dorsal view; C, Urosome, ventral view; D, Antennule. *Scale-bars*: A–C, 100 µm; D, 50 µm

swimming legs (Fig. 3C). Armature on rami as follows (Roman and Arabic numerals indicating spines and setae, respectively).

	Coxa	Basis	Exopod	Endopod
Leg 1	0-0	1-0	I-0; 0-1; II, 5	0-1; II, 5
Leg 2	0-0	1-0	I-0; 0-1; I, 6	0-1; 0-2; I, 4
Leg 3	0-0	1-0	I-0; 0-1; 6	0-1; 0-2; I, 4
Leg 4	0-0	1-0	0-0; 5	0-1; 0-2; I, 3

Leg 1 (Figs. 2D, E, 5F, G) coxa unarmed. Basis with single outer plumose seta. Exopod 3-segmented, with rows of spinules on outer margin of all segments; first segment with small outer spine; second segment with inner plumose seta and a small process (arrowed in Fig. 2D) near base of seta; third segment with small spine on outer corner, long apical spine and 5 plumose setae. Endopod (Fig. 2E) 2-segmented, both segments with rows of spinules on outer margin; first segment about 1.3 times as long as exopodal ramus, with plumose inner seta; second segment with 2 apical spines and 5 setae (one of them tiny located on inner

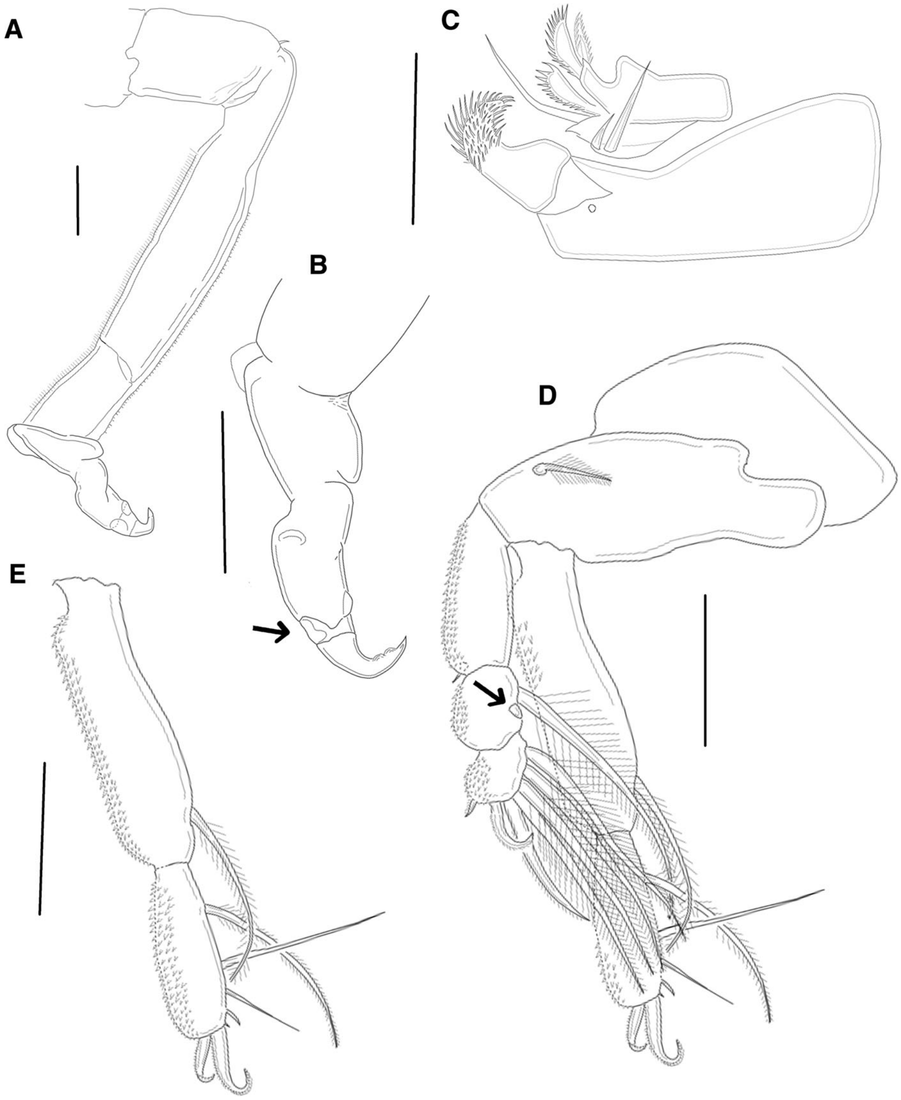

Fig. 2 *Acusicola margulisae* n. sp., holotype female. A, Antenna; B, Distal subchela of antenna, with vestigial third endopodal segment arrowed; C, Mandible, maxillule and maxilla, ventral view; D, Leg 1, anterior view (arrow showing small process at second exopodal segment); E, Leg 1 endopod, anterior view. *Scale-bars*: A, B, 100 µm; C–E, 50 µm

distal corner). Spines of both rami fringed with spinules on outer margin.

Leg 2 short (Fig. 3A) with short outer basipodial seta unarmed. Basis with outer plumose seta. Exopod 3-segmented, with rows of spinules on outer margin of all segments; first segment longest, with outer spine and row of setules on inner margin; second segment with inner plumose seta and small process (arrowed in Fig. 3A) near base of seta; third segment shortest, with minute outer spine and 6 apical plumose setae. Endopod 3-segmented; first segment longest, with row of setules on outer margin and plumose inner seta; second segment with rows of spinules on outer margin and plumose inner setae; third segment with rows of spinules on outer margin, apical spine fringed with spinules on outer margin and setules on inner margin, and 4 plumose setae.

Leg 3 similar to Leg 2, except for the absence of the minute spine on third exopodal segment.

Leg 4 (Fig. 3B) coxa unarmed. Basis with 1 outer plumose seta. Exopod 2-segmented; first segment longest, unarmed, with row of setules on both outer

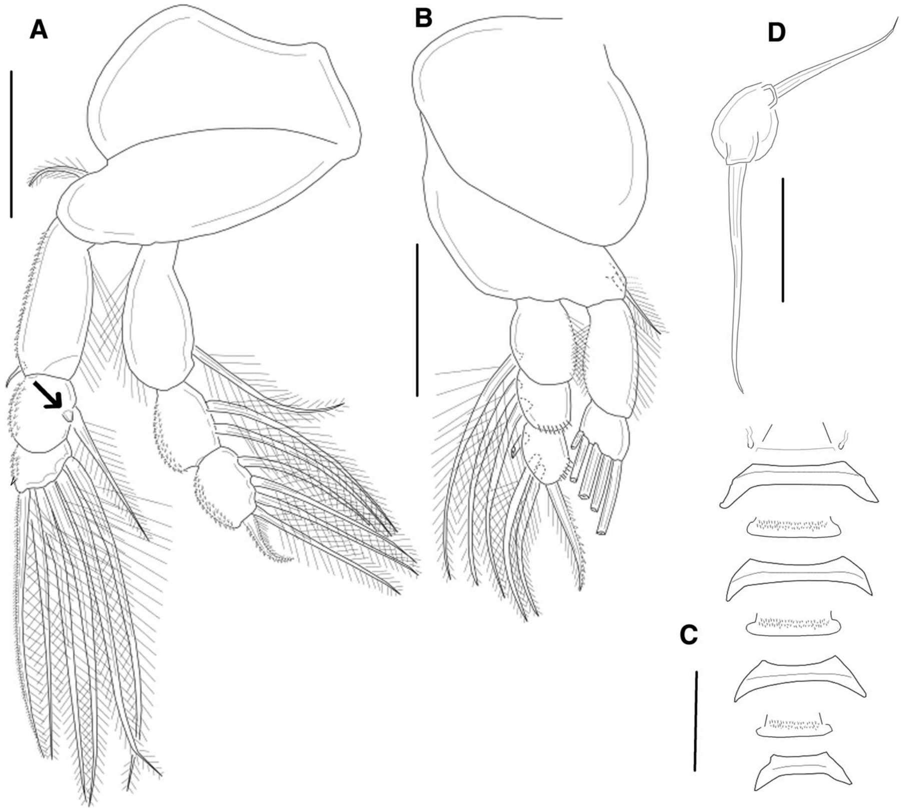

Fig. 3 *Acusicola margulisae* n. sp., holotype female. A, Leg 2, anterior view (arrow showing small process at second exopodal segment); D, Leg 5. *Scale-bars*: A, B, 50 µm; C, 100 µm; D, 25 µm

and inner margins; second segment with 5 long, plumose apical setae (partially drawn in Fig. 3B). Endopod 3-segmented; first segment with row of setules on outer margin and inner plumose seta; second segment with 2 inner plumose setae and row of spinules on distal margin; third segment with row of spinules on distal outer corner, apical spine fringed with spinules on outer margin and setules on inner margin, and 3 plumose setae.

Leg 5 (Fig. 3D) represented by 2 setae; each carried on separate papilla.

Remarks

The new species is distinguished from all known congeners by the relatively longer first endopodal segment of the first leg, being approximately 1.5 longer than second segment, and about 1.3 times as long as exopodal ramus. In the other species of *Acusicola*, the endopodal segments are equally long (e.g. *A. joturicola*, *A. mazatlanesis*, *A. minuta* and *A. spinuloderma* El-Rashidy & Boxshall, 1999), or the first segment is shorter than the second one (e.g. *A. paracunula* Motta Amado & Rocha, 1996 and *A. spinulosa* Motta Amado & Rocha, 1996). The size of setae on the second endopodal segment of the first leg in *A. margulisae* n. sp. also differs from its congeners, particularly the seta located on the inner distal corner, which is much shorter in the new species than in the other species of *Acusicola*. Another characteristic observed only in *A. margulisae* n. sp. is the small inner process on the second exopodal segment of the legs 1-3.

Further, the depression on dorsal surface, between cephalosome and first pedigerous somite, observed in lateral view in the new species, has not been described for any species of *Acusicola*. *Acusicola margulisae* n. sp. most closely resembles four species of *Acusicola*, i.e. *A. tenax* (Roberts, 1965), *A. brasiliensis* da Motta Amado & Rocha, 1996, *A. minuta* Araujo & Boxshall, 2001, and *A. cunula* Cressey, 1970, in having an antenna with elongate first endopodal segment and a

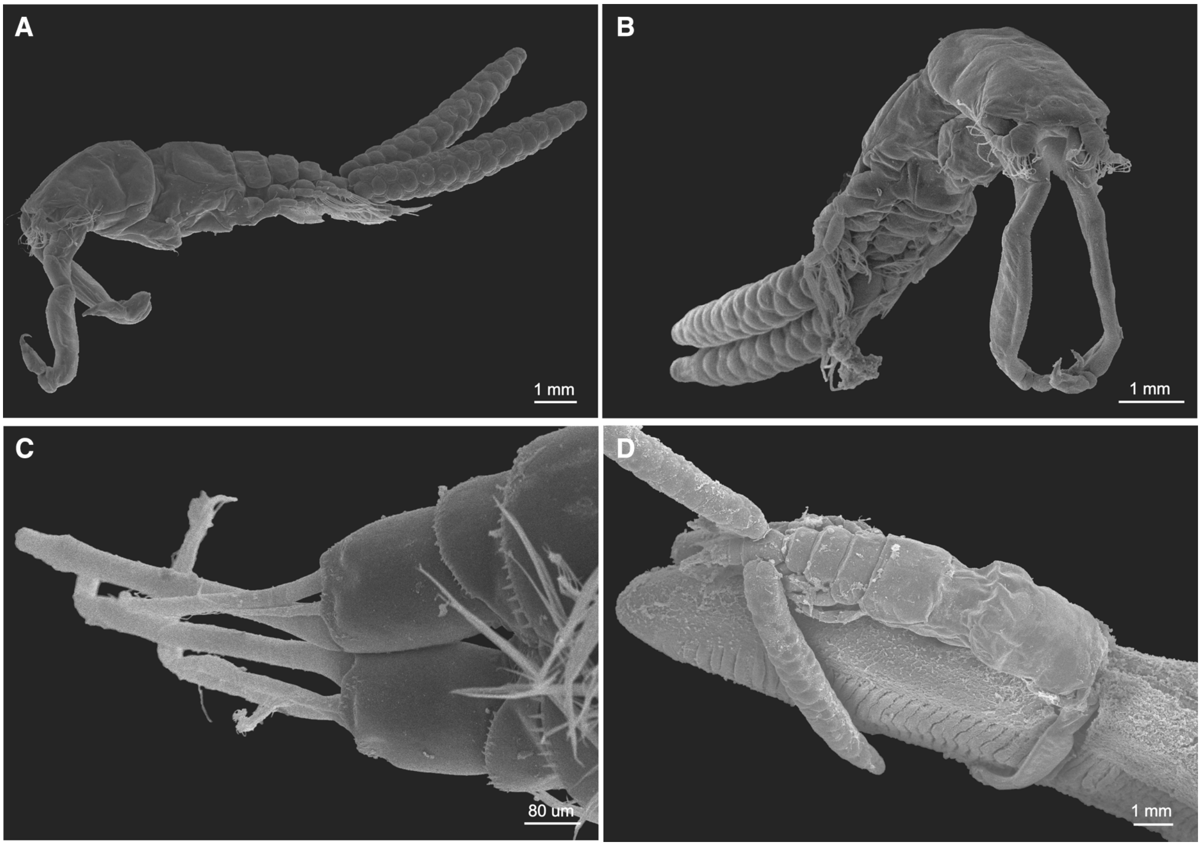

Fig. 4 SEM micrographs of adult female *Acusicola margulisae* n. sp. A, Habitus, lateral view; B, Habitus, ventrolateral view; C, Caudal rami; D, Adult female attached to host gill filament

short distal subchela; a second endopodal segment of first leg with 2 apical spines and 5 inner setae; and the apical spine on third endopodal segment of leg 4 being at least 1.5 times longer than the segment itself (Roberts, 1965; Cressey & Collete, 1970; da Motta Amado & Rocha, 1996; Araujo & Boxshall, 2001). In addition, the indistinct boundary between cephalosome and first pedigerous somite of *A. margulisae* n. sp. is also present in *A. tenax* and in *A. joturicola* El-Rashidy & Boxshall, 1999, *A. lyncengraulidis* Thatcher & Boeger, 1983, *A. mazatlanensis* El-Rashidy & Boxshall, 1999, *A. spinuloderma*, *A. spinulosa* and *A. rotunda* da Motta Amado & Rocha, 1996 (see Roberts, 1965; Thatcher & Boeger, 1983a; da Motta Amado & Rocha, 1996; El-Rashidy & Boxshall, 1999).

Acusicola margulisae n. sp. differs from *A. tenax* and *A. minuta* by having two considerably shorter inner apical setae on the second endopodal segment of leg 1. The new species differs further from *A. tenax* by having one outer spine on the first exopodal segment of leg 1. In addition, Roberts (1965) described the antennule of *A. tenax* as being 6-segmented; however, this needs to be confirmed by examining the type-material. *Acusicola margulisae* n. sp. differs further from *A. minuta* by the absence of two inner membranous expansions on the second endopodal segment of the antenna.

Molecular analysis

The phylogenetic reconstructions using the two nuclear genes yielded *Acusicola margulisae* n. sp. as a member of the family Ergasilidae. The monophyly of the isolates of the new species was well supported based on the evidence of both nuclear markers (18S, Fig. 6; 28S, Supplementary Figure S1). Overall, ML and BI analysis recovered the same topology, with *Acusicola* as the sister taxon of a group of five genera

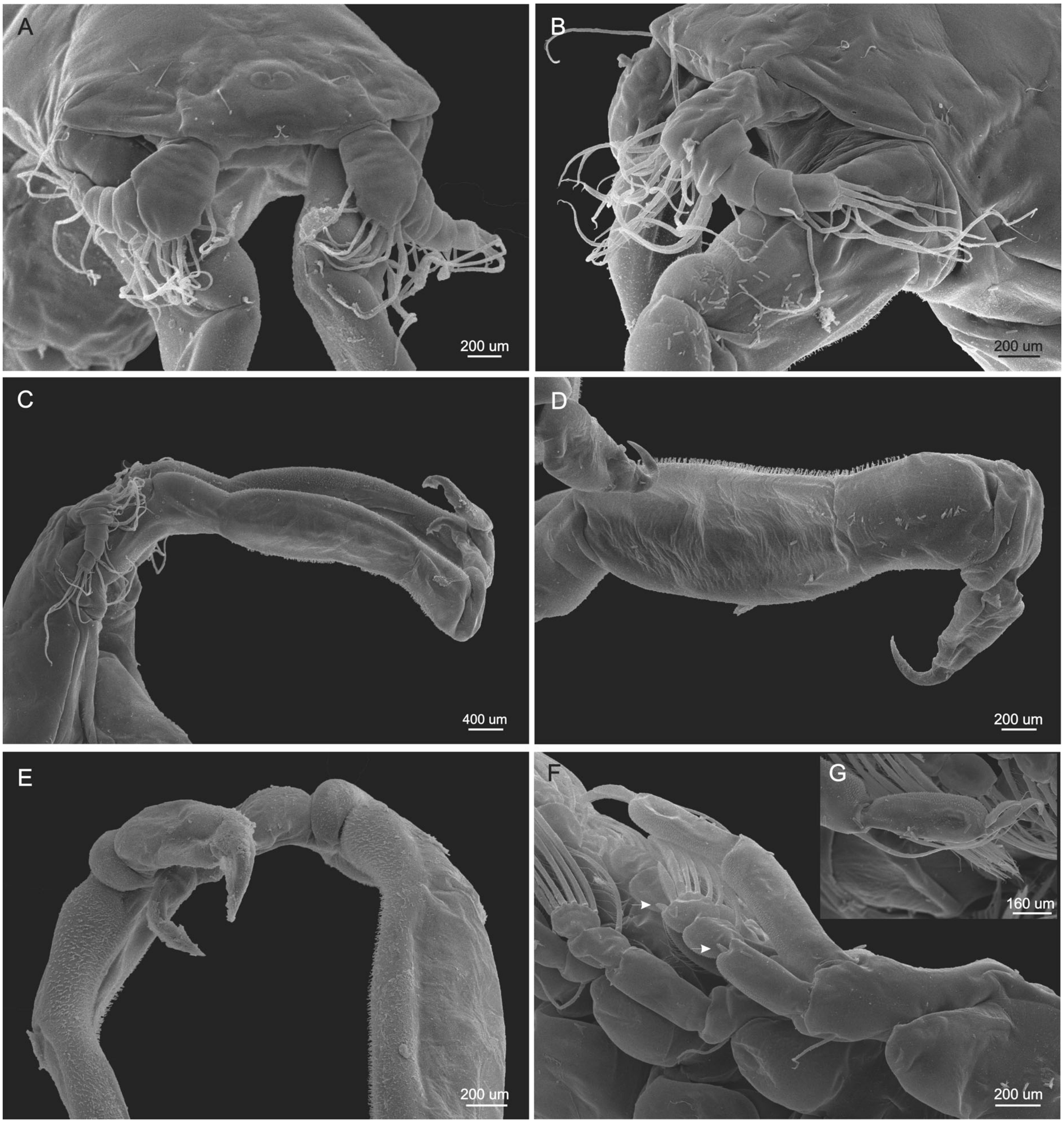

Fig. 5 SEM micrographs of adult female *Acusicola margulisae* n. sp. A, Antennulae, dorsal view; B, Antennule, lateral view; C, D, Antennae, lateral view; E, Antenna, dorsal view; F, Leg 1; G, Distal exopodal segment of Leg 1

of morphologically very similar, i.e. *Ergasilus*, *Pseudergasilus* Yamaguti, 1936, *Paraergasilus* Markewitsch, 1937, *Neoergasilus* Yin, 1956 and *Sinergasilus* Yin, 1942. The genus *Ergasilus* was not recovered as a monophyletic assemblage. The estimated divergence between *A. margulisae* n. sp. and other members of the family Ergasilidae using 18S rDNA ranged between 2.3–5.1% (Supplementary Table S2), and for 28S rDNA divergence ranged between 10.46–18.04% (Supplementary Table S3). The mean intraspecific sequence divergence among 33 isolates of the new species based on *cox*1 sequences was very low (0.4%) indicating a low difference

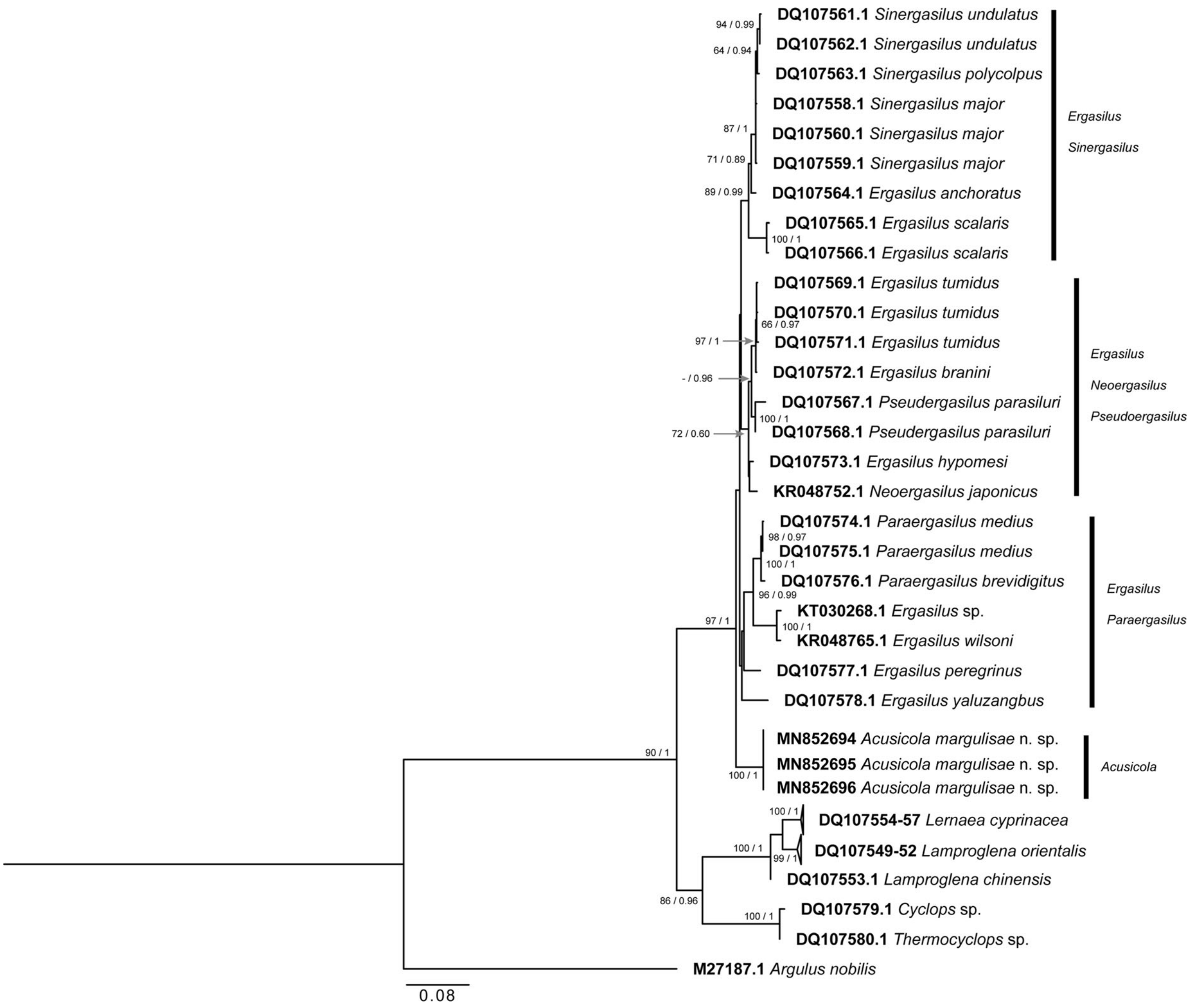

Fig. 6 Maximum likelihood tree for members of the family Ergasilidae, based on 18S rDNA sequences. Bootstrap support and posterior probabilities are displayed at the nodes only if either is over 60%. The scale-bar represents the number of nucleotide substitutions per site

among specimens collected from different host species.

Discussion

The genus *Acusicola* was proposed by Cressey & Collette (1970), with *A. tenax* as the type-species. The genus includes marine and freshwater representatives. Currently, 14 species of *Acusicola* are considered valid, i.e. *A. brasiliensis*, *A. cunula*, *A. joturicola*, *A. lycengraulidis* Thatcher & Boeger, 1983, *A. mazatlanensis*, *A. minuta*, *A. paracunula*, *A. pellonidis* Thatcher & Boeger, 1983, *A. rogeri* Motta Amado & Rocha, 1996, *A. rotunda*, *A. spinuloderma*, *A. spinulosa*, *A. tenax*, and *A. tucunarense* Thatcher, 1984 (see Walter & Boxshall, 2018); all these species have been reported from a range of freshwater, brackish and marine fish hosts, as well as in plankton samples (Table 1). Species of *Amplexibranchius* Thatcher & Paredes, 1985 and *Acusicola* differ from the other ergasilids considered in the study of da Motta et al. (1996) in the structure of the antennae and legs 1-4.

Acusicola margulisae n. sp. is the second species of the genus reported as an ectoparasite of cichlids and the first in poeciliids; *A. tucunarense* was already reported in cichlids from Brazil (Araujo et al., 2009). Members of the family Ergasilidae exhibit low levels

Table 1 Species of *Acusicola* (Copepoda: Ergasilidae) reported in the literature

Species	Host	Habitat	Reference
A. brasiliensis da Motta Amado & Rocha, 1996	*Atherinella brasiliensis* (Quoy & Gaimard) (Atherinopsidae); *Lile piquitinga* (Schreiner, Miranda & Ribeiro) (Clupeidae)	Brackish and marine	da Motta Amado & da Rocha Falavigna (1996)
A. cunula Cressey, 1970	*Pseudotylosurus angusticeps* (Günther) (Belonidae)	Freshwater	Cressey & Collette (1970)
A. joturicola El-Rashidy & Boxshall, 1999	*Joturus pichardi* Poey (Mugilidae)	Brackish	El-Rashidy & Boxshall (1999)
A. lycengraulidis Thatcher & Boeger, 1983	*Lycengraulis grossidens* (Spix & Agassiz) (Engraulidae)	Freshwater	Thatcher & Boeger (1983a)
A. margulisae n. sp.	*Amphilophus citrinellus* (Günther), *Parachromis managuensis* (Günther), *Oreochromis* sp. (Cichlidae); *Poecilia mexicana* (Steindachner) (Poeciliidae)	Freshwater	This study
A. mazatlanensis El-Rashidy & Boxshall, 1999	*Agonostomus monticola* (Bancroft) (Mugilidae)	Brackish	El-Rashidy & Boxshall (1999)
A. minuta Araujo & Boxshall, 2001	Plankton samples	Brackish	Araujo & Boxshall (2001)
A. paracunula da Motta Amado & Rocha, 1996	*Pellona flavipinnis* (Valenciennes) (Pristigasteridae); *Pseudotylosurus microps* (Günther) (Belonidae)	Freshwater	da Motta Amado & da Rocha Falavigna (1996)
A. pellonidis Thatcher & Boeger, 1983	*Pellona castelnaeana* (Valenciennes) (Pristigasteridae)	Freshwater	Thatcher & Boeger (1983b)
A. rogeri da Motta Amado & Rocha, 1996	*Strongylura marina* (Walbaum) (Belonidae)	Freshwater	da Motta Amado & da Rocha Falavigna (1996)
A. rotunda da Motta Amado & Rocha, 1996	*Lycengraulis batesii* (Günther) (Engraulidae)	Freshwater and brackish	da Motta Amado & da Rocha Falavigna (1996)
A. spinuloderma El-Rashidy & Boxshall, 1999	*Agonostomus monticola* (Bancroft), *Joturus pichardi* Poey (Mugilidae)	Freshwater and brackish	El-Rashidy & Boxshall (1999)
A. spinulosa da Motta Amado & Rocha, 1996	*Lycengraulis batesii* (Günther) (Engraulidae)	Freshwater	da Motta Amado & da Rocha Falavigna (1996)
A. tenax (Roberts, 1965)	*Pomoxis annularis* Rafinesque (Centrarchidae)	Freshwater	Roberts (1965)
A. tucunarense Thatcher, 1984	*Cichla ocellaris* Bloch & Schneider (Cichlidae)	Freshwater	Thatcher (1984)

Note: Host names have been updated according to Froese & Pauly (2019)

of host specificity and this is probably the reason why *Acusicola margulisae* n. sp. infects unrelated hosts. The systematics and classification of the genus *Acusicola* have been scarcely studied. da Motta Amado et al. (1995) conducted a cladistic analysis of the family Ergasilidae based on morphological characters. In their study, *Acusicola* was nested as the sister group of the genera *Amplexibranchius* and *Prehendorastrus* Boeger & Thatcher, 1990. More recently, Song et al. (2008) assessed the phylogenetic relationships of 14 species allocated in four of the 24 valid genera of the Ergasilidae; using Bayesian inference in the ribosomal gene 18S. In this study, no representative of *Acusicola* was included. These authors recovered *Ergasilus*, the type-genus of the family as paraphyletic, a result corroborated here. Our study provides the first genetic information and ultrastructural data on the morphology for a species of *Acusicola*.

Yet, the phylogenetic relationships and the current classification of the family Ergasilidae still requires a more comprehensive taxon sampling and a detailed study of the morphology using SEM. More sequences of the 18S rRNA gene, and ideally other ribosomal genes such as 28S rRNA, will be required to accomplish a robust classification system for this important group of parasitic copepods.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the use and care of animals were followed.

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FISH PARASITOLOGY - ORIGINAL PAPER

Taxonomic assessment of the genus *Procamallanus* (Nematoda) in Middle American cichlids (Osteichthyes) with molecular data, and the description of a new species from Nicaragua and Costa Rica

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Abstract

Procamallanus is a species-rich genus of parasitic nematodes of marine, brackish, and freshwater fishes, occurring also occasionally in amphibians and reptiles. In the Neotropical region, this genus is highly diverse, with species described from a wide range of fish families. In this study, we reassess the taxonomic status of *Procamallanus rebecae* with molecular and morphological data and describe a new species endemic to Nicaragua and Costa Rica. We analyzed all *Procamallanus* isolated from fish from the Nicaraguan lakes and some rivers in Costa Rica after an exhaustive analysis of their freshwater fish endoparasite fauna. *Procamallanus rebecae* is a host-specific parasite of Middle American cichlids, previously reported in southern Mexico, Nicaragua, and Costa Rica. We therefore compared these Central American specimens with individuals of *P. rebecae* collected in cichlids from southeastern Mexico using two genomic regions (28S rDNA and mitochondrial cytochrome oxidase subunit 1, COI). We found high levels of sequence divergence between *Procamallanus* from the two geographical regions, with up to 9.8 and 10.5% for both genetic markers, respectively. We also analyzed their morphology and found conspicuous differences in the shape of the mouth and the structure of the female cauda. We therefore describe the specimens of *Procamallanus* from Central American cichlids as a new species. Both *Procamallanus* species occur in different cichlid species and are allopatrically distributed. The host specificity and ancient association patterns between cichlids and *Procamallanus* and the jointly colonization of both hosts and parasites during their northern dispersal from South America are briefly discussed.

Keywords Camallanidae · Parasite · Integrative taxonomy · Cichlids · Host specificity · Biogeography

Introduction

The nematode genus *Procamallanus* Baylis, 1923, is a diverse group of parasites of the family Camallanidae Railliet et Henry, 1915, found in marine, brackish, and freshwater fishes, and occasionally in amphibians and reptiles (Rigby and Rigby 2013). Traditionally, species in this genus have been grouped in five subgenera mainly based on the sclerotization of the oral capsule (Moravec and Thatcher 1997), although recent molecular phylogenetic analyses of camallanids have shown inconsistencies in such classification (Ailán-Choke et al. 2020). *Procamallanus* is very diverse in the Neotropical biogeographical region with up to 30 species described (Ailán-Choke et al. 2018). Four of these species are exclusively found in cichlid fish, two in Middle America belonging to the subgenus *Spirocamallanus* Olsen, 1952, *P. mexicanus* Moravec, Salgado-Maldonado & Caspeta-Mandujano, 2000, and *P. rebecae* Andrade-Salas et al. 1994, and two in South America, belonging to the subgenus *Procamallanus* Baylis, 1923, *P. peraccuratus* Pinto, Fábio, Noronha & Rolas,1976, and *P. spiculastriatus* da Silva, de Vasconcelos, Monks, dos Santos & Giese, 2018. *Procamallanus rebecae* is supposedly distributed throughout Middle America, and has been reported in several cichlid fish species in southeastern Mexico,

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Nicaragua, and Costa Rica (Andrade-Salas et al. 1994; Aguirre-Macedo et al. 2001; González-Solís and Jiménez-García 2006; Sandlund et al. 2010). Due to their close association with cichlid fish, it has been considered part of their biogeographical core parasite fauna (Pérez-Ponce de León and Choudhury 2005).

The intense volcanic activity during the Pleistocene in Central America originated volcanoes that over time collapsed and formed the basis for an array of present crater lakes in this region (Kutterolf et al. 2007). Nicaraguan crater lakes have been thoroughly investigated for their fish fauna (Barlow 1976; Barlow and Munsey 1976; Waid et al. 1999; Elmer et al. 2010a, 2010b), which colonized them from the adjacent great Nicaraguan large lakes, Nicaragua and Managua, of tectonic origin (Barluenga and Meyer 2010). The crater lakes are dominantly inhabited by cichlid fish (Waid et al. 1999), and particularly by the Midas cichlid species complex *Amphilophus* spp. (Barlow 1976). This recent adaptive radiation has been argued to have evolved repeatedly through sympatric speciation (Barluenga et al. 2006; Barluenga and Meyer 2010; Elmer et al. 2010b; Kautt et al. 2016).

During a comprehensive survey of the metazoan endoparasite fauna of fish from Nicaraguan lakes and some rivers in Costa Rica, some specimens of the nematode genus *Procamallanus* were found in the digestive tract of cichlid fish. We analyzed these nematodes together with *P. rebecae* collected in cichlids from southeastern Mexico. In order to elucidate the phylogenetic relationships of these nematode parasites, they were compared using morphological and molecular approaches.

Materials and methods

Sample collection

Fish were collected across three parasitological surveys carried out in Nicaragua, in November–December of three consecutive years, 2017–2019. Fish were sampled from the great Nicaraguan Lakes Nicaragua and Managua, and from five crater lakes in the surrounding region. The parasitological scanning was carried out on a total of 896 fish, including 20 cichlid species and seven species of non-cichlid fish. We studied non-cichlid fishes in the locality to further corroborate that the species is indeed a cichlid parasite in the geographic area. Additionally, we included specimens of *Procamallanus* from two species of cichlids (18 individuals) sampled in Costa Rica in 2015. We also included samples of *Procamallanus* obtained from cichlids collected between 2003 and 2014 in eight localities of southeastern Mexico (Fig. 1, Table 1). All fish were collected with gill nets, cast nets, and harpooning, then sacrificed with an overdose of tricaine (MS-222), dissected, and all organs immediately studied for parasites under a stereomicroscope. We isolated all *Procamallanus* specimens, rinsed them in 6.5% saline solution, and fixed in 100% EtOH or 4% hot (nearly boiling) formalin for molecular and morphological analyses, respectively.

Molecular analyses

Genomic DNA was isolated using DNAzol Reagent (Invitrogen) or Speedtools tissue DNA extraction kit (Biotools) according to the manufacturer's protocol, from a fragment of the middle body of individual nematodes (hologenophore *sensu* Pleijel et al. 2008). We amplified two molecular markers, the mitochondrial cytochrome oxidase subunit 1 (COI) and the large subunit of the 28S rDNA. The partial COI region was amplified using the primers 507 (5′-AGTTCTAATCATAARGATATYGG-3′) (Nadler et al. 2006) and HCO (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). The partial 28S rDNA region was amplified using the primers 391 (5′-AGCGGAGGAAAAGAAACTAA-3′) and 536 (5′-CAGCTATCCTGAGGGAAAC-3′) (García-Varela and Nadler 2005). The amplification and sequencing protocols followed those used in Santacruz et al. (2020). Sequences were assembled and edited using Geneious v7 (Kearse et al. 2012).

A dataset for each genetic marker was constructed including the newly generated sequences plus sequences of camallanids available in GenBank. The species *Dracunculus lutrae* Crichton and Beverly-Burton, 1973, was used as outgroup based on previous phylogenetic analyses (Černotíková et al. 2011; Choudhury and Nadler 2016). Sequences were aligned with the T-COFFEE platform (http://tcoffee.crg.cat; (Di Tommaso et al. 2011). The best-fit model of molecular evolution for each dataset was calculated with ModelFinder (Kalyaanamoorthy et al. 2017) using the Bayesian information criterion (BIC). Phylogenetic reconstruction for each marker was performed with maximum likelihood (ML) in IQ-TREE v.1.6.2 (http://iqtree.cibiv.univie.ac.at/), with Ultrafast bootstrap; 10,000 iterations, SH-aLTR branch test; 10,000 replicates (Nguyen et al. 2015; Hoang et al. 2017). Phylogenetic reconstruction was also run applying Bayesian inference (BI) in MrBayes v3.2.7a (Huelsenbeck and Ronquist 2001), using the CIPRES Science Gateway Web Portal v3.3 (Miller et al. 2010). Analyses were performed using two runs of four chains, each for 10^7 generations, sampling trees every 2000 generations, and a temperature of 0.2. The first 25% of trees were discarded as burn-in and the resulting trees were used to obtain a 50% majority-rule consensus tree. Tracer v1.7.1 (Rambaut et al. 2018) was used to assess convergence of independent runs, with effective sample sizes (ESS) > 200. The pairwise distances between groups for each dataset were calculated as uncorrected *p-distances* in MEGA v7 (Kumar et al. 2016).

Table 1 List of sampling localities of *Procamallanus* spp. in Middle American cichlids, with geographical coordinates. Abbreviation codes are used in Figs. 1, 2, and 3

Species	Country	Locality	Code	North	West
Procamallanus barlowi n. sp.	Costa Rica	River Irigaray, Liberia	RIL	10° 43′ 21″	85° 30′ 36.99″
		River Las Animas	RAC	11° 02′ 58″	85° 35′ 12″
	Nicaragua	Lake Apoyo	APO	11° 56′ 12.23″	86° 02′ 58.95″
		Lake Apoyeque	AYE	12° 14′ 41.32″	86° 20′ 24.81″
		Lake Asososca León	ASL	12° 26′ 4.03″	86° 39′ 54.61″
		Lake Masaya	MAS	11° 56′ 57.40″	86° 07′ 37.70″
		Lake Managua (Xolotlán)	MAN	12° 22′ 04.92″	86° 22′ 43.79″
		Lake Nicaragua (Cocibolca)	NIC	11° 10′ 05.23″	84° 58′ 44.08″
Procamallanus rebecae	Mexico	Sinkhole Non Choncunchey, Yucatán	CNC	20° 48′ 53″	90° 11′ 47″
		El Zapote, Campeche	EZ	19° 16′ 55.35″	90° 36′ 44.53″
		Gregorio Méndez, Chiapas	GM	17° 27′ 01.97″	91° 35′ 48.37″
		Lake San Pedro Tenosique, Tabasco	LSP	17° 38′ 46.99″	91° 22′ 58″
		Metzabok, Chiapas	ME	17° 07′ 3.41″	91° 37′ 54.61″
		River Champotón, San Antonio del Río, Campeche	RCA	19° 19′ 13.32″	90° 33′ 21.52″
		Salto Grande, Candelaria, Campeche	RGC	18° 11′ 46.01″	91° 07′ 10.48″
		San Isidro, Centla, Tabasco	SIC	18° 22′ 59.59″	92° 35′ 55.83″

Morphological analyses

The hologenophores and complete individuals were cleared in a glycerol-alcohol solution (1:1) to observe and measure internal structures using an Olympus BX51 light-inverted microscope equipped with differential interference contrast (DIC) optical components. To study the external ultrastructure, specimens were prepared for scanning electron microscopy (SEM). Samples were sonicated, critical point dried, mounted on a strip of carbon conductive tape, and then coated with gold. The SEM photographs were taken in a Hitachi scanning electron microscope 15 kV Hitachi Stereoscan Model SU1510. Type material was deposited in the Colección Nacional de Helmintos (CNHE), Institute of Biology, UNAM, México.

Results

We analyzed a total of 265 *Procamallanus* specimens, 231 isolated from 13 cichlid species in the Nicaraguan lakes, 18 isolated from two cichlid species from rivers in northwestern Costa Rica, and 16 specimens from five cichlid species in water bodies in Mexico.

Molecular analyses

We obtained sequences from two genomic regions. All obtained sequences were deposited in GenBank (accession numbers COI: MW487868–MW487887, 28S rDNA: MW485586–MW485594). The 28S rDNA phylogenetic reconstruction distinguished two well-supported *Procamallanus* lineages from Middle American cichlids, a relationship supported by moderate to high bootstrap and posterior probability values (80/0.98) (Fig. 2). One clade corresponded to previous descriptions of *P. rebecae*, and the second clade represented a well-differentiated lineage, arguably a new species. Both are reciprocally monophyletic and exhibit very high level of genetic divergence for this molecular marker, with an average of 9.8% (Table 2). The phylogenetic reconstruction with COI also resolved two well-differentiated lineages, *P. rebecae* and the new species, each forming a well-supported monophyletic clade (Fig. 3). The genetic divergence between both lineages averaged 10.5% (Table 2). Additionally, intraspecific genetic divergence within clades was very low for COI, (0.57% *P. rebecae*, 0.63% for the new species). The two lineages were not resolved as closely related to two other species of *Procamallanus* from Middle America from which sequences are available, i.e., *P. neocaballeroi* Caballero-Deloya, 1977, and *P. gobiomori* Moravec, Salgado-Maldonado & Caspeta-Mandujano, 2000. Both 28S rDNA and COI trees corroborated the findings of other studies in that the genus *Procamallanus* is paraphyletic. The topology of the trees is not comparable since the sequences available in GenBank for each molecular marker include different species. Since both trees revealed that the isolates of *Procamallanus* sampled in cichlids from Nicaragua and Costa Rica represent independent evolutionary units, a detailed morphological analysis was performed, and the new species is described next.

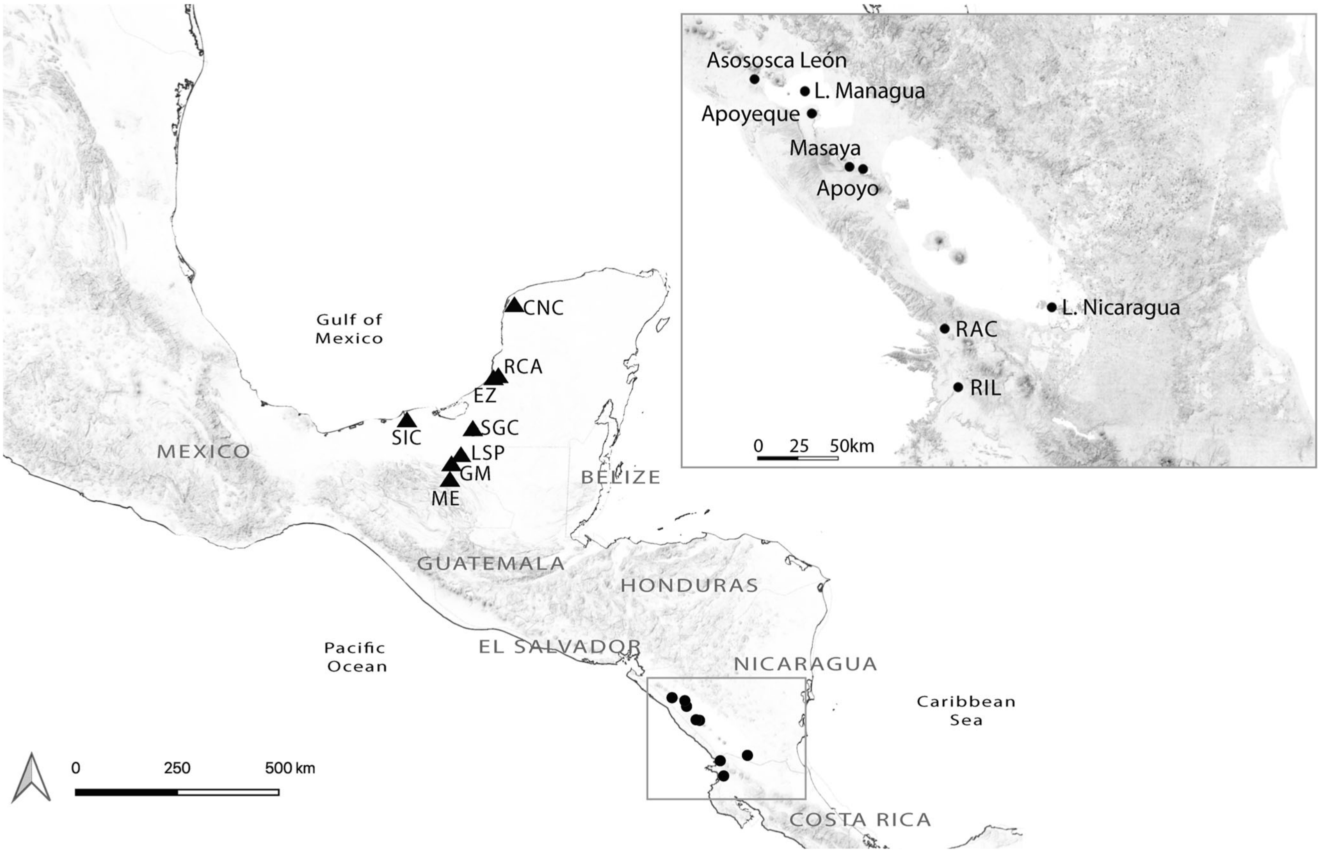

Fig. 1 Map showing the sampling sites of *Procamallanus* spp. in cichlid hosts in Middle America, with a close-up to the great lakes and crater lakes of Nicaragua. Locality code according to Table 1

Family Camallanidae Railliet & Henry, 1915

Genus *Procamallanus* Baylis, 1923

Subgenus *Spirocamallanus* Olsen, 1952

***Procamallanus barlowi* n. sp.**

Type host: *Amphilophus chancho* Stauffer, McCrary & Black

Type locality: Lake Apoyo, Nicaragua (11° 56′ 12.239″ N, 86° 2′ 58.956″ W)

Other localities: **Nicaragua**: Lake Apoyeque: *Parachromis managuensis* (Günther); Lake Apoyo: *Amphilophus astorquii* Stauffer, McCrary & Black, *Amatitlania nigrofasciata* (Günther), *Amphilophus zaliosus* (Barlow), *P. managuensis*; Lake Asososca León: *Amphilophus citrinellus* (Günther); Lake Managua: *Amphilophus labiatus* (Günther), *Parachromis* sp.; Lake Masaya: *Cribroheros longimanus* (Günther); Lake Nicaragua: *Amphilophus citrinellus*, *Amphilophus labiatus*, *Cribroheros longimanus*, *Cribroheros rostratus* (Gill), *Hypsophrys nicaraguensis* (Gill), and *Hypsophrys nematopus* (Günther). **Costa Rica**: River Irigaray: *Cribroheros*

Table 2 Uncorrected *p-distances* shown as percentages, COI distance below the diagonal, and 28S distance above the diagonal. In bold, the COI intraspecific distance of the two *Procamallanus* species in cichlids

	Species	1	2	3	4	5	6	7	8
1	*Procamallanus barlowi* n. sp.	**0.63**	9.14	20.55	25.08	32.50	24.63	25.40	25.81
2	*Procamallanus rebecae*	10.49	**0.57**	20.72	25.37	33.57	25.40	26.98	25.50
3	*Procamallanus neocaballeroi* species complex	13.51	13.93	–	22.69	29.88	22.59	24.30	27.53
4	*Batrachocamallanus slomei*	13.83	15.96	16.27	–	22.20	3.73	14.95	29.01
5	*Camallanus kaapstaadi*	13.98	14.45	15.19	14.31	–	23.30	19.49	35.61
6	*Batrachocamallanus xenopodis*	14.09	16.04	16.44	0.00	14.29	–	15.30	29.15
7	*Procamallanus pseudolaeviconchus*	15.36	15.08	15.22	15.56	15.25	15.42	–	27.93
8	*Spirocamallanus huacraensis*	17.79	17.94	20.74	21.45	19.95	21.45	19.38	–

longimanus, River Las Animas: *Amatitlania nigrofasciata* (Günther).

Site of infection: Intestine and stomach.

Prevalence and abundance: Fig. 4.

Type material: Holotype (male) CNHE 11472, allotype (gravid females) CNHE 11473 (Lake Apoyo), and paratypes CNHE 11474 (Lake Apoyo), Nicaragua.

Representative DNA sequences: COI: MW487868–MW487887, 28S rDNA: MW485586–MW485594.

ZooBank registration: urn:lsid:zoobank.org:act:59F70A20-1366-4AE3-81A2-E83781CC71AD

Etymology: the species is named after George Barlow who was a Professor of ichthyology and animal behavior at UC Berkeley, not only for his numerous contributions to cichlid biology but also for his passion and enthusiasm that inspired many generations of cichlid specialists.

Description (Figs. 5 and 6, measurements in Table 3)

General: Medium-sized nematodes, with slightly striated cuticle. Mouth opening oval, surrounded by 12 papillae arranged in three circles (Fig. 5c); four papillae on each circle (Fig. 6a, b). Papillae of external circle larger. Papillae of innermost circle with a pore close to the edge of the mouth (Fig. 6c). One pair of lateral amphids present. Well-developed basal ring (Fig. 6d). Orange-brown buccal capsule, with thick walls, lining internally with 13–15 spiral thickenings; first three anterior spirals incomplete (Fig. 5a). Spiral thickenings with

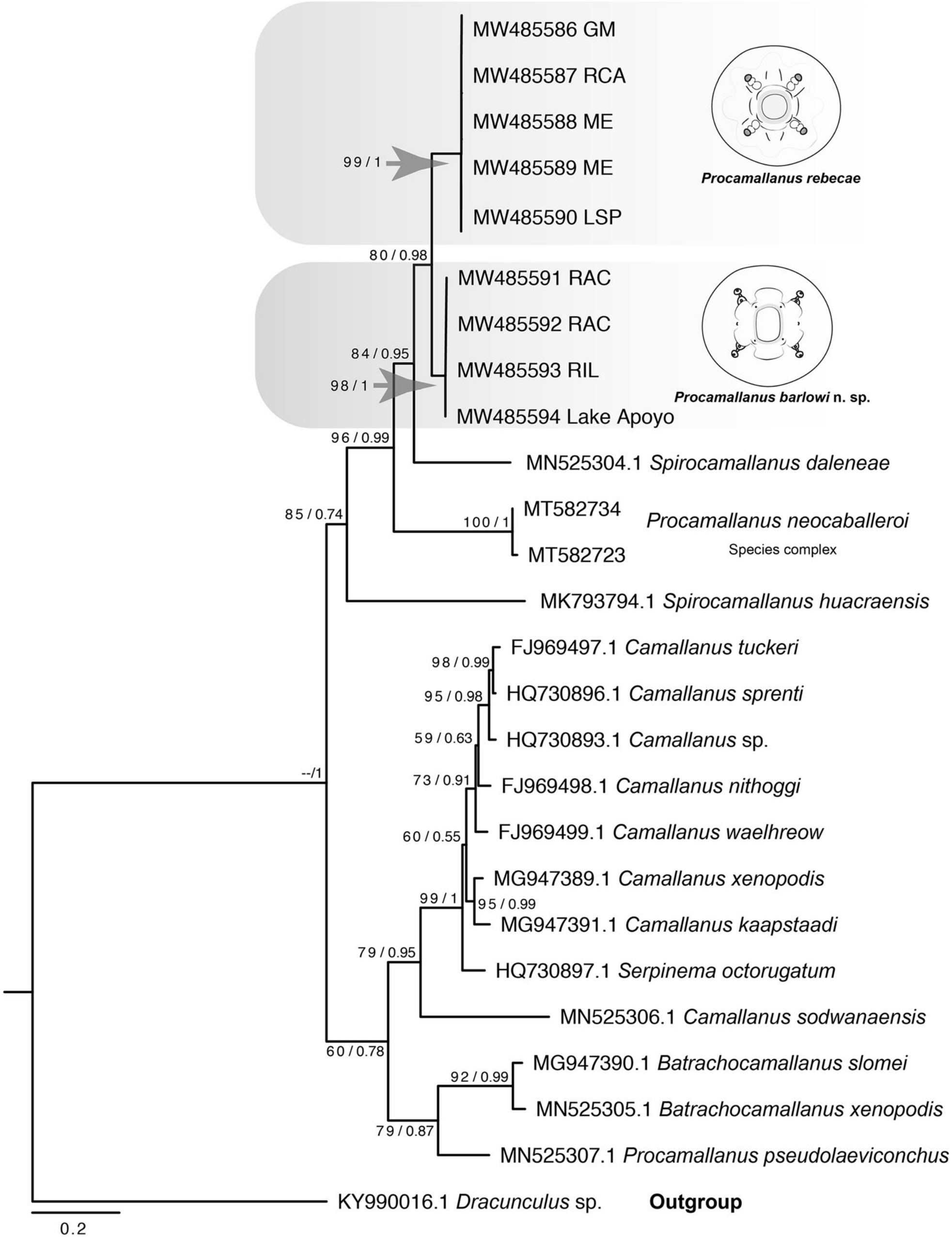

Fig. 2 Consensus tree from IQ-TREE analysis of the 28S rDNA matrix of *Procamallanus* spp. Best-fit model according to BIC: TIM2+F+G4. Node support values are from ML bootstrap and Bayesian posterior probability, respectively. The drawings correspond to apical views of *P. rebecae* and *P. barlowi* n. sp.

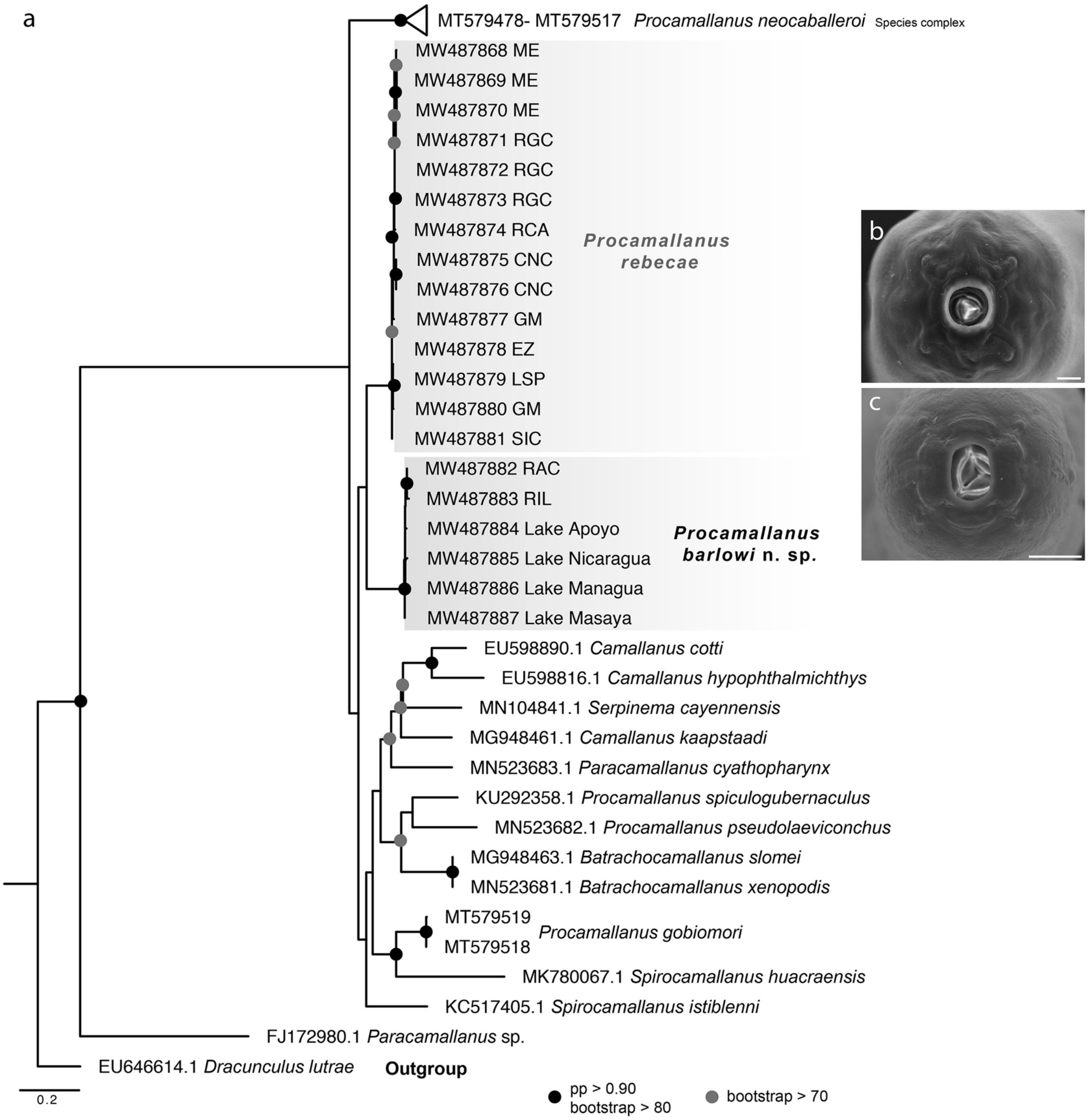

Fig. 3 Consensus tree from IQ-TREE analysis of the COI matrix (**a**). Best-fit model according to BIC: TPM3+F+I+G4. The number in each node indicates the posterior probability from the BI analyses and the size of the circle is equivalent to the bootstrap support from the ML. **b**, **c** Anterior end of *P. rebecae* and *P. barlowi* n. sp. through SEM, respectively. The scale bar is equivalent to 15 µm

smooth edges (Fig. 6c), limited to two-thirds of buccal capsule in both sexes (Fig. 6a). Small and simple deirids (Fig. 6e). Nerve ring surrounding first half of muscular esophagus (Figs. 5a, b). Excretory pore in last third of muscular esophagus (Figs. 5a and 6g).

Female: Vulva equatorial (Fig. 5f), vulval lips not elevated. Phasmids in ventrolateral position, half-way between anus and caudal tip (Fig. 6h). Papillae surrounding anal aperture absent (Fig. 6i). Tail rounded, with terminal digit-like projection. Caudal tip with three small cuticular extensions in gravid and mature females (Fig. 5e).

Male: Smaller than female. Spicules unequal, left spicule longer than right spicule (Fig. 5g). Gubernaculum absent. Caudal alae with nine pairs of pedunculate papillae (Figs. 5g and 6j–n). Three pairs of subventral preanal papillae, and six pairs of postanal papillae; first four pairs subventral and last two pairs lateral; last pair probably representing phasmids. Two pairs of sessile adcloacal papillae. Three cuticular spine-like extensions on tip tail (Fig. 6m). In male juveniles, walls of the buccal capsule more thickened than in mature males and caudal alae fully developed with only one spicule observed.

Differential diagnosis

The new species belongs in *Procamallanus* in having a buccal capsule lined with continuous walls, internally smooth or with markings in one sex or both. In total, six species of

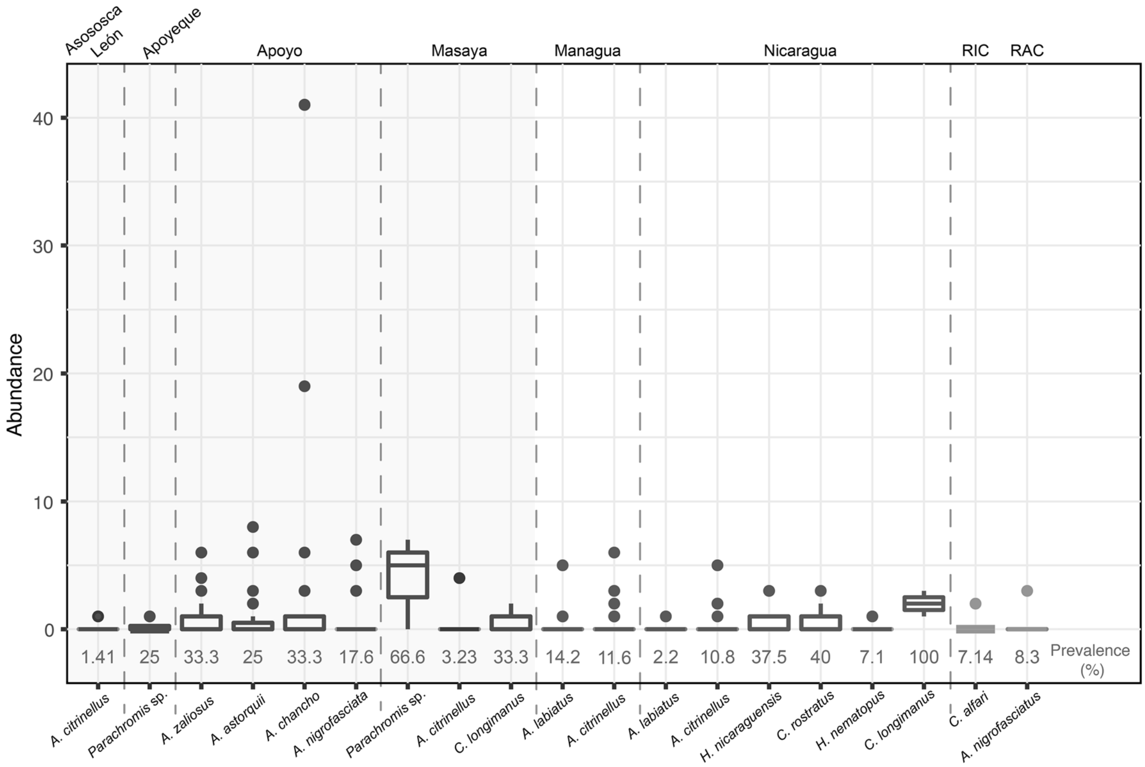

Fig. 4 Prevalence and abundance of *Procamallanus barlowi* n. sp. Only positive infections in each host-site combination are shown. On the gray background, the infection profiles of the cichlids in the crater lakes are shown

Procamallanus distributed in Middle American freshwaters are considered valid, all within the subgenus *Spirocamallanus*, namely *P. gobiomori*; *P. jaliscensis* Moravec, Salgado-Maldonado & Caspeta-Mandujano, 2000; *P. mexicanus*; *P. neocaballeroi*; *P. pereirai* (Anneraux, 1946) Olsen, 1952; and *P. rebecae* (see Garrido-Olvera et al. 2006). Two of these species of *Procamallanus* are parasites of cichlids*:* *P. mexicanus*, described from *Cichlasoma gedessi* (Regan) (= *Herichthys gedessi*) in Xalapa, Veracruz, Mexico, and *P. rebecae*, originally described from *C. helleri* (Steindachner) (= *Thorichthys helleri*) in Campeche, Mexico, and currently distributed in 13 cichlid species in Mexico (Garrido-Olvera et al. 2006), and four cichlid species in Nicaragua and Costa Rica (Aguirre-Macedo et al. 2001; González-Solís and Jiménez-García 2006; Sandlund et al. 2010). For comparison, Fig. 7 depicts SEM photomicrographs of *P. rebecae*. The new species morphologically resembles these two species by the presence of three preanal papillae; however, *P. barlowi* n. sp. differs in the number of spiral ridges lining the buccal capsule and a different external shape of the mouth (Figs. 6b and 7a); furthermore, the female of *P. barlowi* n. sp. possesses a rounded cauda bearing a digit-like projection, whereas this projection is conical in *P. rebecae*; the female of the new species also possesses spikes on the tip of the tail which are absent in the female of *P. mexicanus*.

Two additional species of *Procamallanus* have been reported in cichlids from Brazil, both within the subgenus *Procamallanus*: *P. spiculastriatus* and *P. peraccuratus*. These two species are readily distinguished from the new species by the presence of an internally smooth capsule in males and females. In addition, *P. spiculastriatus* exhibits a ring with tooth-like structures and lacks spine-like projections on the tip of the tail. Interestingly, in African cichlids, only one species of *Procamallanus* (*Spirocamallanus*) has been reported, *P. serranochromis* Moravec and Van As 2015 infecting species of *Serranochromis* spp. (Moravec and Van As 2015). This species shares several morphological features with *P. barlowi* n. sp. such as the presence of three pairs of preanal papillae, wide caudal alae, and asymmetrical spicules; nevertheless, this species can be differentiated from *P. barlowi* n. sp. by having a bilobed tip tail in both sexes instead of spike-like as in the new species.

In Middle American non-cichlid freshwater fishes, three more species of *Procamallanus* (*Spirocamallanus*) have been described, i.e., *P. neocaballeroi* from characids, *P. gobiomori* from eleotrids, and *P. jaliscensis* from mugilids. All these species have a buccal capsule completely filled with spiral grooves, while the new species lacks spiral ridges in the last third of the buccal capsule. The new species shares with four additional Neotropical species of *Procamallanus* (*Spirocamallanus*) the presence of spiral thickenings in the

Table 3 Measurements of morphological characters of *Procamallanus barlowi* n. sp. Measurements expressed as the mean followed by the range (in parenthesis); all measurements are in μm, unless otherwise stated

Morphological features	Males	Gravid females	Non-gravid females
Body L	12.253 (10.134–13.273) mm	-	-
Body W	236.670 (205.003–289.714)	233.891 (179.059–288.723)	247.220 (210.323–284.117)
Buccal capsule L	68.831 (64.138–75.661)	68.845 (64.258–69.378)	62.915 (53.542–73.969)
Buccal capsule L including basal ring	75.857 (70.321–81.181)	73.573 (68.283–78.271)	69.498 (57.790–82.164)
Buccal capsule W	55.600 (53.121–56.689)	59.946 (58.213–63.203)	52.606 (44.053–58.403)
Basal ring L	32.009 (28.745–34.101)	36.161 (32.577–42.910)	32.180 (27.445–37.739)
Basal ring W	6.720 (5.157–6.781)	6.620 (5.798–7.612)	5.582 (5.483–5.772)
Number of spiral rings in the oral capsule	13–15	13–15	13–15
Length of the area covered with spiral grooves lining the buccal capsule	57.445 (51.908–63.471)	52.641 (50.674–54.608)	50.195 (40.355–60.311)
Muscular esophagus L	357.819 (327.332–373.363)	379.822 (352.606–408.316)	350.818 (316.545–375.723)
Muscular esophagus W	58.661 (49.94–66.736)	65.280 (53.365–77.119)	57.416 (44.346–64.101)
Glandular esophagus L	737.612 (572.737–811.195)	658.613 (556.977–735.814)	569.646 (449.324–742.451)
Glandular esophagus W	79.400 (44.726–101.659)	73.108 (57.822–98.200)	69.620 (56.145–81.017)
Muscular/glandular esophagus length ratio	1:1.74–1:2.17	1:1.57–1:1.80	1:1.41–1:1.97
Nerve ring from anterior extremity	99.044 (97.061–100.948)	210.982 (202.805–219.836)	212.373 (191.429–231.312)
Deirids from anterior extremity	-	101.021 (95.539–106.504)	91.825 (82.568–101.082)
Excretory pore from anterior extremity	-	387.957	238.772
Right spicule L	304.860	-	-
Left spicule L	202.766	-	-
Spicules length ratio	1:1.5		
Tail L	-	216.851	230.667 (219.038–242.297)
Vulva from anterior extremity	-	6.276 (5.262–7.289) mm	5.695 mm
Body of mature larvae L	-	-	-
Body of mature larvae W	-	-	-
Number of terminal spine-like	3	0–3	0–3

second third of the oral capsule in both females and males, namely *P. belenensis* Giese, Santos & Lanfredi, 2009; *P. inopinatus* Travassos, Artigas & Pereira, 1928; *P. saofranciscensis* Moreira, Oliveira & Costas, 1944; and *P. pintoi* Kohn & Fernandes, 1988. However, all these species are readily distinguished from the new species because they lack caudal alae and possess short spicules.

Discussion

In the present work, we describe a new species of endoparasite, the nematode *Procamallanus barlowi* n. sp., found in Central American cichlids. Therefore, the previous taxonomic records of *P. rebecae* from Nicaragua and Costa Rica (Aguirre-Macedo et al. 2001; González-Solís and Jiménez-García 2006; Sandlund et al. 2010) correspond with this newly described species. The new species is widely distributed throughout the River San Juan basin, including Nicaragua's Great lakes, nearby crater lakes, and rivers in northwestern Costa Rica, displaying high host specificity to cichlid fish. Whether or not this species of nematode is also found in cichlids in other areas of lower and nuclear Central America requires further exploration. At the moment, both species are regarded as allopatrically distributed, and we acknowledge that there is a geographic gap in our samples to define if there is a transition area between both species and their cichlid hosts where they might occur in sympatry.

The new species herein described following an integrative taxonomy approach and *P. rebecae*, form a strongly supported monophyletic clade that infects specifically Middle American cichlids, and can be regarded as a part of their biogeographical core parasite fauna (*sensu* Pérez-Ponce de León and Choudhury 2005). The genetic divergence with both mitochondrial and nuclear markers between these two species is well above the distance threshold observed within other *Procamallanus* lineages (Santacruz et al. 2020), and also above that reported for other nematode species (e.g., Solórzano-García et al. 2020; Chen et al. 2020). Furthermore, the close evolutionary relationship between

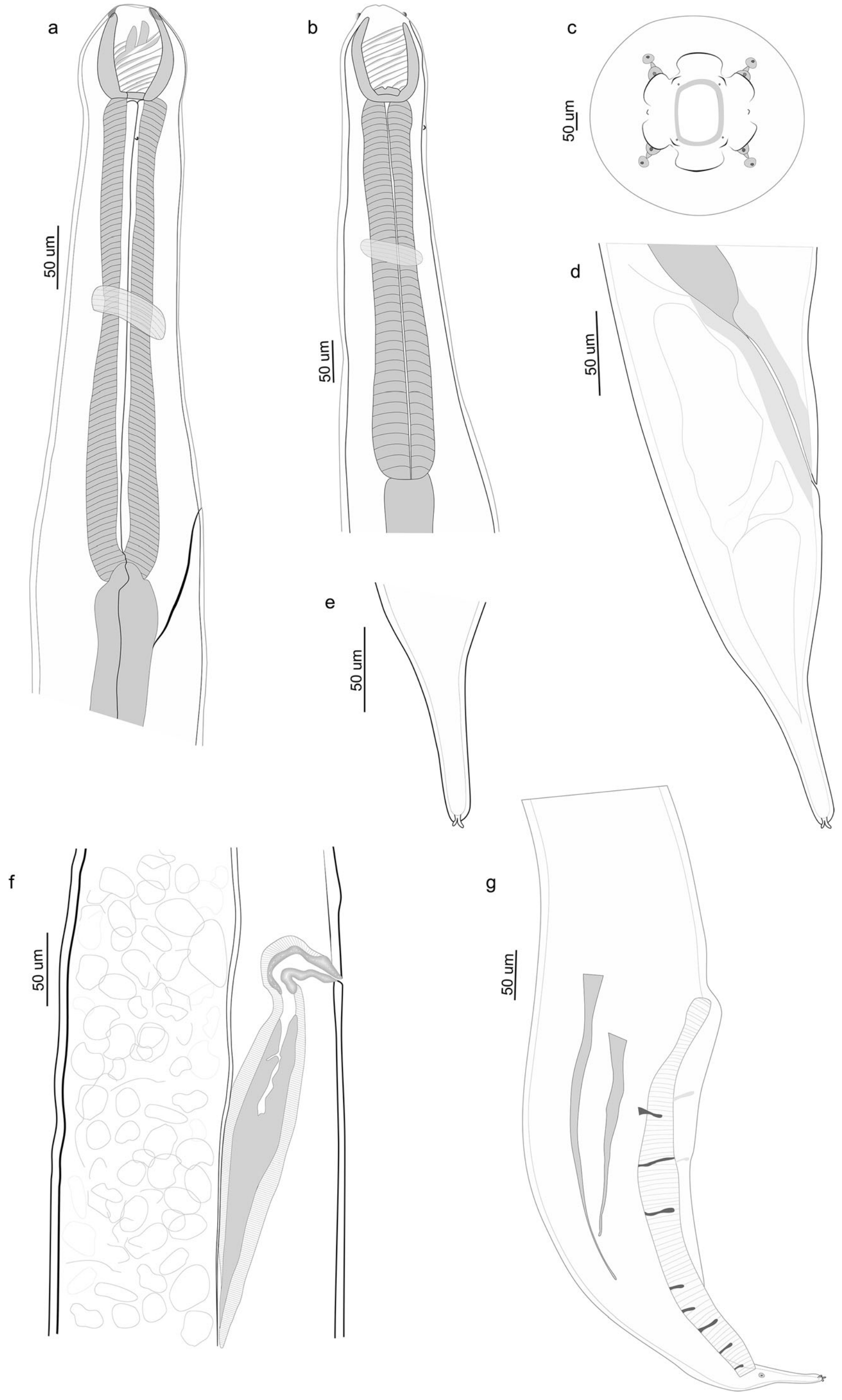

Fig. 5 *Procamallanus barlowi* n. sp. **a** Anterior end of female, lateral view. **b** Anterior end of male, ventral view. **c** Anterior end, apical view. **d** Female tail, lateral view. **e** Tail tip of female. **f** Vulva. **g** Posterior end of male, lateral view

P. barlowi n. sp. and *P. rebecae*, the geographic scenario and host association, shows that the parasites of cichlids may have diversified along with their hosts, during the northward dispersal and colonization of new freshwaters from South America. Several empirical studies have demonstrated an extensive geographical range for some cichlid parasite lineages across Middle America, e.g., *Crassicutis cichlasomae* Manter, 1936, and *Sciadicleithrum* spp. (Kritsky, Thatcher, and Boeger, 1989; Mendoza-Franco and Vidal-Martínez 2005; Razo-Mendivil et al. 2010), whereas other studies have proven speciation events of both associates related with the northern dispersal after the closure of the Panama Isthmus. This pattern seems to be also common since some species pairs of helminths in cichlids reflect sister-species relationships between species found in the Central American cichlid assemblages and those of southeastern Mexico, e.g., *Oligogonotylus manteri* Watson, 1976–*Oligogonotylus mayae* Razo-Mendivil, Rosas-Valdez & Pérez-Ponce de León, 2008, and

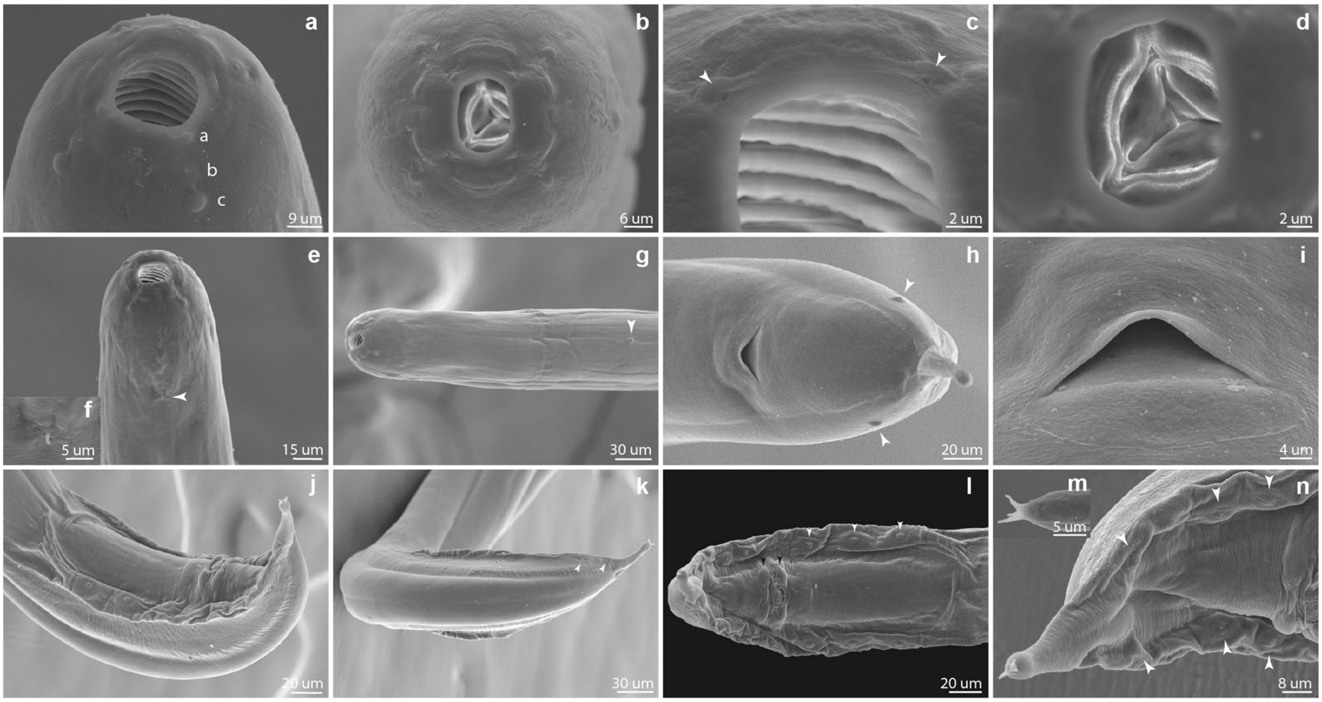

Fig. 6 Scanning electron microscopy photomicrographs of *Procamallanus barlowi* n. sp. **a**, **b** Anterior end, lateral and apical view, respectively. **c** Male apical view, the arrows indicate the pores at the edge of the mouth. **d** Basal ring. **e** Position of the deirid in a lateral view. **f** Deirid. **g** Excretory pore position indicated by the arrowhead. **h** Female tail with a pair of lateral phasmids indicated by the arrowheads. **i** Female anal aperture. **j** Male tail, ventrolateral view. **k** Male tail, dorsal view; posterior lateral papillae indicated by the arrowheads. **l** Male tail, ventral view; right preanal papillae and sessile adcloacal papillae indicated by the white and black arrowheads, respectively. **m** Spine-like structures in the male tail tip. **n** Postanal papillae in the mail tail indicated by the arrowheads. Abbreviations: **a**, **b**, **c** cephalic papillae of internal, middle, and external circle, respectively

Neoechinorhynchus costarricense Pinacho-Pinacho et al. 2020–*Neoechinorhynchus golvani* Salado, 1978 (Razo-Mendivil et al. 2008; Pinacho-Pinacho et al. 2020). Our study provides further support for this pattern.

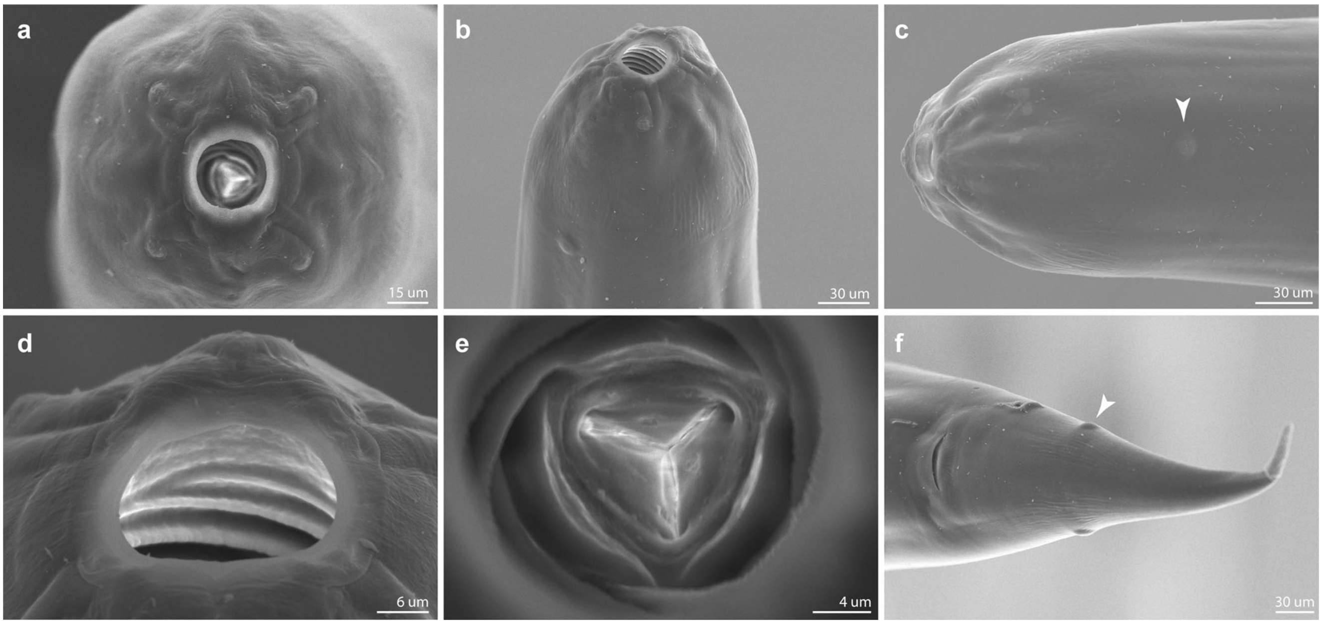

Fig. 7 Scanning electron microscopy photomicrographs of *Procamallanus rebecae* female. **a** Apical view of the anterior end. **b** Anterior end, ventrolateral view. **c** Position of the deirid in a lateral view. **d** Inner surface of the buccal ridges. **e** Basal ring. **f** Tail, with phasmids indicated by the arrowheads

Our molecular phylogenetic hypotheses point out towards an ancient association between cichlids and *Procamallanus* which is probably older than their arrival to Middle America, since both species are not closely related with other *Procamallanus* from freshwater fish occurring in the same geographic area. With this data, we hypothesize that the South American *Procamallanus* from cichlids could be clustered with *P. rebecae* and the new species and form a clade that has evolved tightly linked to their cichlid hosts. However, in order to adequately test for this hypothesis, it would be required to collect additional data of *Procamallanus* in cichlids, and to combine this analysis with data from the only species of the genus reported in African cichlids, which would allow evaluating transcontinental dispersal. Such approach would be very useful to further understand the historical biogeography of cichlids and their parasites (Pariselle et al. 2011; Vanhove et al. 2016). Within the American continent, cichlids dispersed from South America into Middle America as a result of several waves of colonization (Říčan et al. 2013, 2016). Cichlids followed their northern dispersal and were able to expand further north, into rivers, lakes, and sinkholes (in the Yucatan Peninsula) to reach the boundaries between the Neotropical and Nearctic biogeographical zones as other fish groups such as characids (and their *Procamallanus*) (Ornelas-García and Pedraza-Lara 2016; Pérez-Miranda et al. 2018; Santacruz et al. 2020). Our study also evidenced that including additional data of *Procamallanus* from South American freshwater fishes would be required to trace the evolutionary patterns of both hosts and parasites. Likewise, the wide distribution of *P. barlowi* n. sp. in cichlids of the lake system of Nicaragua, a model system for the study of island-like colonization (Elmer et al. 2010a), makes the new species an ideal example to study the tempo and mode of colonization in contrast to their hosts.

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Declarations

Ethics approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest The authors declare no competing interests.

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CAPÍTULO 4. Los parásitos de los peces en los lagos de Nicaragua

Los cíclidos son la fauna dominante en los lagos de Nicaragua, y comprenden numerosas especies endémicas, principalmente del complejo de especies del cíclido de Midas. La diversidad y grado de endemismo está ligado a su aislamiento en los lagos cráter. Debido a que los datos parasitológicos son escasos, enfocamos nuestra investigación en estos peces y caracterizamos la diversidad y distribución de su fauna de parásitos. Encontramos una riqueza conformada por 37 taxones de parásitos, principalmente de helmintos (Artículo III). La riqueza se distribuye de acuerdo con un modelo continente-isla, mayor número de especies en los lagos grandes que en los lagos cráter más pequeños y aislados. Sorprendentemente, algunos lagos cráter albergan una gran diversidad de parásitos no compartidos con el resto de los lagos. El cíclido de Midas alberga una fauna núcleo de parásitos conformada por ocho taxones, cada uno con biologías únicas.

En este trabajo analizamos peces de otras familias que coexisten con los cíclidos de Midas, aunque con un esfuerzo de muestreo significativamente menor. Esto se hizo con la finalidad de abordar la riqueza única o compartida con los cíclidos. Adicionalmente, recuperamos de la literatura todos los registros parasitológicos en peces de Nicaragua, y de los países que conforman la denominada América Central Baja (Lower Central America) (Costa Rica y Panamá) (Artículo IV). En conjunto con nuestros resultados, encontramos una fauna de parásitos de 101 taxones descritos en menos del 50% de las especies de peces de agua dulce que se distribuyen en el país. La mayor parte de las especies son trematodos. El reciente esfuerzo de muestreo posiciona a Nicaragua como el país con más especies de parásitos registradas, aún cuando su riqueza de peces es menor que Costa Rica y Panamá.

Este capítulo constituye la base de conocimiento para abordar el objetivo principal de este estudio. Además, abrió el panorama para derivar el resto de las preguntas de investigación. El capítulo está integrado por los siguientes manuscritos:

4.1 Los parásitos de cíclidos en los lagos de Nicaragua

Artículo III

Santacruz A, Barluenga M & Pérez-Ponce de León, G. (2022) First steps towards understanding host-parasite diversification and speciation in a model system: macroparasite diversity in cichlid fish from Nicaraguan lakes. *Scientific Reports* (*in press*).

4.2 Los parásitos de peces de agua dulce de Nicaragua

Artículo IV

Santacruz A, Barluenga M & Pérez-Ponce de León G. (2022) Filling the knowledge gap of Middle American freshwater fish parasite biodiversity: Metazoan parasite fauna of Nicaragua. *Journal of Helminthology* (*in press*).

Macroparasite diversity in cichlid fish from Nicaraguan lakes: first steps towards understanding host-parasite diversification and speciation in a model system

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Abstract

The Nicaraguan lakes, including the great lakes and several surrounding crater lakes, comprise an ideal continent-island-like setting to study the colonization patterns of both fish and their parasites. The dominant fish fauna of this system are cichlids, and particularly the recent radiations of Midas cichlid species complex *Amphilophus* spp. This well-studied model for recent sympatric speciation provides an excellent model for studying patterns of host-parasite evolution in allopatric and sympatric settings. Here, we characterized the fish macroparasite diversity in the great Nicaraguan lakes and several crater lakes, with emphasis on the Midas cichlid. We evaluated patterns of parasite diversity across host populations. Morphological and molecular analyses were conducted, revealing that the macroparasite fauna was composed by 37 taxa, most of them already described in other Middle American cichlids. Parasite diversity includes representative species of platyhelminths, nematodes, copepods, branchiurans, hirudineans and oribatid mites. Among these parasites, three invasive species are reported for the first time. The Midas cichlid was infected by 22 parasite taxa, 18 shared with other cichlids. The core parasite fauna of the Midas cichlid was present in all populations and lakes with few exceptions, and was composed by eight parasite taxa. The large lakes had larger parasite diversity than the smaller and more isolated crater lakes. However, parasite infracommunity diversity was not larger in the great lakes. This is the first comprehensive survey of cichlid fish parasites in the Nicaraguan lakes, which provides a ground to explore the evolutionary history of the parasites within the context of the speciation and diversification processes of both hosts and parasites.

Keywords: parasites; biogeography; core parasite fauna; cichlids, parasite richness.

Introduction

Parasitism is one of the most common ecological interactions in nature[1]. Parasites inform about the ecology and evolutionary history of their hosts[2]. Notwithstanding of the potential detrimental effect on their hosts, parasites display important roles in ecosystems since they exert strong selective pressures on host populations, regulating their populations in multiple ways[3], determining the presence of other parasites[4], modulating host behaviors[5–7], and influencing host range expansion[8]. In this context, changes in parasite community composition and abundance might be an evolutionary driving force for their host populations[9–12]. The first step in understanding the structure of parasite communities and the role of these communities on hosts and ecosystems, is to collect comprehensive knowledge on parasite species composition. Besides, hosts and parasites may be the result of a long evolutionary history, and may play an important role on each other's diversification processes[13].

The Nicaraguan lakes system comprise two large tectonic lakes (the great Nicaraguan lakes, Managua and Nicaragua) and several young adjacent crater lakes, establishing a set-up analogous to a continent-island model. The fish fauna of this region is dominated by cichlids and poecilids, and the largest biomass corresponds to the Midas cichlid species complex (*Amphilophus* spp*.*)[14]. The Midas cichlid independently colonized each of the crater lakes in the last few thousand years by fish stocks from the great Nicaraguan lakes[15–17]. Therefore, the Midas cichlid is the outcome of recent adaptive radiations through sympatric and allopatric speciation[15,18–20]. The endemic species dwelling each crater lake display great intra-specific variation in color, body shape and trophic traits[21–23], and recurrent phenotypes with evident evolutionary parallelisms across lakes[24]. However, the information about the parasite fauna of fish in this geographical area, and particularly in cichlids representing a model system, is still scarce.

There has been an intense effort in the Neotropics to document the freshwater fish parasite diversity. About 200 species of parasites have been described in neotropical cichlids[25]. However, a gap in the parasitological knowledge remains for Central American freshwater fishes[26], including the Nicaraguan lakes. The most important contribution was published back in 1976[27] with the description of the digenean fauna of fishes from Lake Nicaragua. Very few posterior studies reported fish parasites in some Nicaraguan streams of both the Pacific and Atlantic slopes[28–33]. In total, 71 species of metazoans have been reported in Nicaraguan freshwaters, but only 19 in the great Nicaraguan lakes[27,34] and surrounding crater lakes[35]. Our recent investigation has already resulted in the description of two additional species of parasites from Nicaraguan crater lakes[36,37], and more are on the way.

The objectives of this study were two-fold: first, to assess the diversity of macroparasites in cichlid fishes of Nicaraguan lakes, the dominant fish group in the area, and second, to characterize the spatial distribution of parasite communities among host species and lakes, in the search for parasite diversity patterns and the processes that determine them. We expect to find heterogeneous patterns of parasite distribution among lakes, associated to different fish community composition and to different historical contingencies in each lake.

METHODS

Fish collection and parasitological survey. Fish were collected from the two great Nicaraguan lakes, Managua and Nicaragua, and five crater lakes (Asososca León, Apoyeque, Xiloá, Masaya and Apoyo) during the months of November-December of three consecutive years (2017-2019). Fish were caught with gill nets, anaesthetized with tricaine mesylate (MS-222) and photographed on a lateral standardized position for species identification. Fish were euthanized in cold water and fin clips were preserved in ethanol. Ecto- and endoparasites were screened in the field immediately after euthanization with a Leica EZ4 stereomicroscope. First, the fish external surface (skin, eyes, and mouth) was assessed. Then, fish were dissected, and internal organs (gut, mesentery, muscle and gall, swim, and urinary bladders) were analyzed. All recovered parasites were rinsed in saline solution and stored in 100% ethanol. Representative specimens of each parasite taxa were fixed in nearly boiling 4% formalin for morphological analysis. Some specimens were fixed in 100% ethanol for DNA extraction. Gill arches were dissected and stored in 100% ethanol. Later, in the laboratory, the gills were screened for ectoparasites using a Leica EZ4 stereomicroscope.

Morphological characterization of parasites. All parasites were characterized morphologically to achieve their taxonomic identity. Parasites fixed in formalin were rinsed in distilled water and dehydrated in increasing ethanol concentrations (from 10 to 70%). Whole specimens of monogeneans, or partial sections of the body (anterior/posterior voucher regions of the body) were mounted in semi-permanent preparations of ammonium picrate- glycerin. In some cases, the haptor region or the male copulatory organ was enzymatically digested with proteinase K to recover only sclerotized structures, and then mounted on a Gray & Weiss solution for permanent preparations. Additionally, monogeneans, cestodes, trematodes and hirudineans were stained with Mayer´s paracarmine or Gomori´s trichrome and mounted on permanent slides using Canada balsam. The remaining parasite taxa, including nematodes, copepods, branchiurans and oribatid mites were cleared with a glycerin/alcohol solution (1/1) and mounted as semi-permanent slides. All specimens were photographed with an Olympus BX51 inverted light microscope equipped with differential interference contrast (DIC) optics. Voucher specimens of parasites were deposited in national collections housed at the Biology Institute, National Autonomous University of Mexico (UNAM), Mexico City, i.e., Colección Nacional de Helmintos (CNHE), Colección Nacional de Crustáceos (CNCR), and Colección Nacional de Ácaros (CNAC) (Supplementary Table S1). Additionally, the ultrastructure of the external surface of some individual parasites was analyzed through scanning electron microscopy (SEM). Individuals were dehydrated, critically point-dried, mounted on a strip of carbon conductive tape, coated with a thin layer of gold, and observed in a Hitachi SEM unit SU1510.

For further investigation of taxonomically problematic groups, specimens fixed in 100% ethanol were individually sequenced to corroborate their identification or to reveal potential cryptic diversity. For example, mitochondrial DNA sequences of the cytochrome oxidase subunit II (cox2) were obtained for larval stages of the nematode *Contracaecum* spp. which are difficult to distinguish morphologically.

The phylogenetic position, nodal support and genetic distance were estimated for some parasite taxa to establish more robust species limits, and on some occasions to establish conspecificity. The molecular markers used were the mitochondrial cox1, cox2, and the nuclear genes 18S, and 28S, depending on the genetic library available for each parasite group (Supplementary Table S2). All generated sequences were submitted to GenBank (Supplementary Table S1).

Data analysis. Parasite communities were described at the infracommunity level, i.e., all the parasite taxa occurring within an individual host within a locality[38]. This allowed us to compare all the parasite communities occurring in different host species in a single lake, but also to compare all the communities among the seven sampled lakes. The three sampling seasons were considered replicates and were pulled together; for ectoparasites, calculations only included data from two sampling seasons (2017 and 2018). The characterization of each infracommunity was based on the following ecological parameters: species richness, abundance, and diversity through Shannon-Wiener and Simpson diversity indices. Species richness is the number of parasite taxa harbored by an individual host. Abundance is the number of individuals of all parasite taxa sampled by individual host[39]. The Shannon-Wiener diversity index estimates species diversity and their uncertainty; increasing values of the index reflect increasing diversity and evenness. Instead, Simpson diversity index estimates the richness and relative abundance of species[40]. These indices were calculated using the *diversity* function in the package ‘vegan‘ in R[41]. To test the significance of parasite diversity among host species and among lakes, the non-parametric Kruskal-Wallis (KWT) and Wilcoxon tests were implemented using R[42]. In order to identify the most common parasites, prevalence of infection (proportion of hosts infected by a given parasite taxon) was used to generate a heatmap of interactions with the R package ‘ggplot2’[43].

We analyzed the representativeness of our sample for describing parasite species richness in this region with rarefaction and extrapolation curves using the R package ‘iNEXT’[44]. This approach employs Hill numbers (i.e., effective number of species). Hill numbers are a family of diversity indices that employ species richness and relative abundance, differing between them by an exponent q[45]. In our study we use the order of species richness (with $q = 0$). Individual hosts represent the sampling units, and the number of parasite detections through all the sampling units represents the incidence data. We used 100 bootstrap iterations to generate 95% confidence intervals for the rarefied and extrapolated curves. All the analysis were conducted in R[42].

RESULTS

Macroparasite fauna. A total of 754 cichlid fish were studied for parasites in seven Nicaraguan lakes, belonging to 20 fish taxa, 10 of which corresponded to the Midas cichlid species complex (509 individuals) and formed the largest portion of the sample (Supplementary Table S3). Midas cichlids are particularly abundant in these lakes. The majority of hosts (n = 717, 94.34%) in all lakes were infected by at least one parasite taxa. We recovered over 70,000 parasites, representing 37 taxa, of which 16 were identified to species level (Fig. 1, Table 1). One fourth of these species were already described in the region. Considering the whole parasite community, the lakes contributed unequally to the total species richness. The great lakes Nicaragua and Managua hold the highest parasite richness, with 21 and 16 parasite taxa, respectively, and the crater lakes had lower parasite species richness, ranging from 5 to 14 taxa (Fig. 2, Supplementary Table S4). Some parasite taxa were restricted to a few lakes. Hirudineans were only found in Lake Nicaragua, oribatid mites were found in crater Lake Xiloá and cestodes were found solely in the great lakes and crater Lake Xiloá. Copepods were absent from crater Lake Apoyeque and Masaya. The remaining parasite taxa were found in all lakes.

Parasites were mainly represented by helminths, either as larval forms or as adults, because fish serve as the intermediate or definitive host (Table 1). Trematodes and nematodes were the most abundant and diverse parasite groups. Cestodes and oribatid mites were rare. Three invasive species were found, including the African monogenean *Cichlidogyrus sclerosus*, the Asian copepod *Lernaea cyprinacea*, and the Asian cestode *Schyzocotyle acheilognathi*. Twenty-two of the 37 parasite taxa were found in the Midas cichlid complex. The most common parasites in the Midas cichlid were the trematodes *Crassicutis cichlasomae*, *Oligogonotylus manteri*, and *Saccocoelioides* spp., the nematodes *Contracaecum* spp. and *Procamallanus barlowi*, the copepod Ergasilidae gen. sp., the monogenean *Sciadicleithrum mexicanum,* and the acanthocephalan *Neoechinorhynchus costarricense*, forming the Midas cichlid core parasite fauna. This core fauna was also shared with cichlids of the genus *Parachromis*. These common parasites were widely distributed in the host populations, although consistently absent from some particular crater lakes. For instance, the nematode *P. barlowi* was absent in fishes from crater Lake Xiloá, whereas the monogenean *S. mexicanum* was absent in Midas cichlids from crater lakes Asososca León and Apoyeque, but it was present in *Parachromis* spp. in both lakes.

We analyzed 20 host species patchily distributed among lakes, making a total of 41 host-lake combinations. The distribution of the 37 parasite taxa was heterogeneous, with some of them present in a range between one and 29 combinations (Fig. 3). The most widely distributed parasites were the trematodes *C. cichlasomae* and *O. manteri*, and the nematodes *Contracaecum* spp. and *Hystherothylacium* sp. The heatmap depicted in Fig. 3 also showed that most parasite taxa differed largely in prevalence of infection, with some reaching values of 100%.

Infracommunity structure among lakes and hosts*.* Overall, parasite species richness per individual host ranged from zero to 8 species; richness ranged between zero and 7 and between zero and 4 for endoparasites and ectoparasites, respectively. On average, the observed richness at the infracommunity level was 2.71 species, whereas richness was 1.83 and 1.20 for endoparasites and for ectoparasites, respectively. Parasite infracommunities among lakes were significatively different in richness (KWT, $p < 0.001$), Shannon-Wiener (KWT, $p < 0.001$), and Simpson (KWT, $p < 0.001$) diversity indices (Table 2). Parasite infracommunities from crater Lake Apoyo were the most diverse and evenly distributed, deviating most from the rest of lakes. The least diverse communities where those in the extremely isolated crater Lake Apoyeque, although these were also evenly distributed according to the Shannon-Wiener diversity index. The large lakes had similar infracommunity richness to those of crater lakes Asososca León, Xiloá and Masaya. According to the Shannon-Wiener diversity index, crater lakes Xiloá and Masaya had the most uneven infracommunities. The Simpson diversity index revealed no major differences among lakes.

We analyzed the parasite infracommunities of the Midas cichlid species complex among and within lakes (Table 3). Infracommunity richness was largest in *A. zaliosus* from crater Lake Apoyo, and smallest in *A. citrinellus* from crater Lake Apoyeque. The Midas cichlids in crater Lake Apoyo had in general larger richness than in the rest of lakes. Within lakes, Midas cichlid species differed in diversity indices. Within the large lakes, the lipped species *A. labiatus* consistently had larger parasite infracommunity richness and higher Shannon-Wiener index values than its congener *A. citrinellus* (Wilcoxon, L. Managua $p < 0.001$ and L. Nicaragua $p = 0.019$, respectively). Within the crater lakes, limnetic species (*A. zaliosus* in crater Lake Apoyo and *A. sagittae* in crater Lake Xiloá) had larger richness than other benthic species, although these differences were not significant ($p > 0.05$).

Rarefaction and extrapolation curves for species richness indicated that sampling size was representative for the Midas cichlid complex (Fig. 4) to describe the structure of metazoan parasite infracommunities, with the exception of some rare species in crater Lake Apoyo, and in crater Lake Masaya, where more samples from *A. citrinellus* would improve the sample. The extrapolated curves suggested that expected parasite richness was highest in the great Nicaraguan lakes, Nicaragua and Managua confirming the rest of results (Table 2, Table 3).

DISCUSSION

Parasites represent a large fraction of the Earth´s total biodiversity[1]. It has been estimated that, they account for something between one-third to over half of the species on the planet[46]. Cichlid fish are a model system in evolutionary biology due to their vast diversity and spectacular diversification rates, particularly those of the East African Great lakes[47], but their parasite faunas still remain understudied. Cichlid parasites provide a great opportunity for exploring the role of host-parasite coevolution in adaptive radiations, and can even provide information about their biogeography and dispersal history[25]. Our study represents the first comprehensive survey of the metazoan parasite fauna of cichlids in the Nicaraguan lakes. Emphasis was put on the Midas cichlid species complex (*Amphilophus* spp.) because they represent a model system in evolutionary biology for their recent, repeated, and often sympatric adaptive radiations[15,18–20,23,48,49]. Here, we provide the first host-parasite records for some crater lakes and their cichlid hosts.

Our inventory of the macroparasites infecting cichlid fish in the Neotropical region of the Nicaraguan great lakes identified 37 parasite taxa in 20 cichlid taxa, and a subset of 22 in the Midas cichlid species complex. This duplicates the information existing for the lake's region and considerably increases the information on this species complex. The most common parasites were endoparasites (60% of the parasites found), and the most common taxa were trematodes and nematodes. This agrees with what has been shown for other Neotropical cichlids[50], but contrasts with the records reported for African cichlids, where the most common parasites found so far are ectoparasites (monogeneans), while nematodes, and particularly trematodes, are considerably less common[9–12,25,51]. Even if we are aware of geographic and taxonomic bias in the study of cichlid parasites (much greater interest on ectoparasites in Africa), the data supports a very marked historical signature of parasite communities in both African and American flocks[52].

Previous studies have shown that each fish family possess their own set of parasites with tight taxonomical associations[50,53], but the environment also shapes parasites communities, forming characteristic regional or biogeographic faunas[50,54,55]. Cichlids occurring in Nicaraguan freshwaters harbor a parasite community typical of the Neotropical region. The core parasite fauna of the Midas cichlid consisted of at least three trematodes, two nematodes, one acanthocephalan, one copepod, and one monogenean. These species are relatively common among cichlids occurring in Central America[28–30], and some species, e.g. *Oligogonotylus manteri* is found in cichlids further north in southeastern Mexico[56]. Moreover, trematodes were the most diverse and common parasites and, *Crassicutis cichlasomae* was particularly abundant.

The Nicaraguan lakes form a set-up analogous to a continent-island model and, in accordance with this model, the great Nicaraguan lakes which resemble the continent, hold larger parasite diversity than in the smaller and younger crater lakes. The larger size, connections to surrounding rivers, and larger number of cichlid and non-cichlid species, might explain these differences. Interestingly, infracommunity diversity was not larger in the great lakes. On average, each fish had three parasite taxa, two of them endoparasites, and one ectoparasite. Parasite infections reached intensities as high as over 750 monogeneans, 1000 trematodes or 1800 larval nematodes in individual hosts. To the best of our knowledge, these intensity levels of parasite infections have not been reported in cichlids before.

Crater Lake Apoyo hold the richest parasite infracommunities with 3.5 parasites per individual fish, and within the Midas cichlid, four parasites per fish. Crater Lake Apoyeque hold the poorest parasite infracommunities with only two parasites per fish. Within this extremely isolated lake, no copepods were found, and in the Midas cichlid only two trematodes and one acantocephalan were found (see Supplementary Table 4). Crater Lake Masaya also had low infracommunity parasite diversity (2.5 parasites per fish), and we also found no copepods in this lake. In crater Lake Masaya rarefaction and extrapolation curves suggest that sample sizes might be insufficient to adequately describe their macroparasite fauna. It is interesting to note that another very isolated crater lake (Asososca León), despite having a very impoverished fauna, possess several unique parasites that increase richness values (average of three parasites per fish). In this lake we found two nematode species, that despite intense sampling effort were not found in any other lake. These nematodes, that infect fish as larval stages, complete their life-cycle on piscivorous birds, their definitive hosts, that should easily disperse them across the region, potentially interconnecting populations of different lakes[57]. It does not seem to be the case for these parasites. In crater Lake Asososca León, the Midas cichlid was not infected with monogeneans and, instead, these cichlids harbored a large number of copepods on the gills.

The very parasite diverse crater Lake Apoyo, another crater lake with severely impoverished fish fauna, a cichlid fish was recently introduced, the convict *Amatitlania nigrofasciata*, from a nearby crater lake (MB pers. obs.). This cichlid was introduced in 2018 and in our sample only harbors three parasite species, all of them common cichlid parasites. On average, compared to populations of this species in other lakes, no significant difference of parasite infracommunity diversity was found. However, it is noticeable the infection of convicts by the nematode *P. barlowi*, very abundant in Midas cichlids in crater Lake Apoyo, and very rare in any other lake. This observation has to be taken with caution due to limitation of sampling size of convict cichlids in some of these localities. However, this might constitute a gain of a new parasite after invasion, although additional samples would be required to confirm this hypothesis. Introduced species might be released from their natural enemies and therefore perform better in the new environment[58]. In addition, after colonization of a new environment invaders might acquire generalist parasites from other hosts occurring in the same area. This has been shown in helminth parasites transmitted by birds of introduced convict cichlids in a Mexican river[59]. The African tilapia is a cichlid introduced in Nicaraguan waters several decades ago[60]. We studied the macroparasites of this species in two Nicaraguan lakes, and found some African parasites that do not seem to have been transmitted to native cichlids (e.g., the monogenean *Cichlidogyrus sclerosus*). As recently shown by[61] the fact that exotic parasites can be co-introduced with tilapia may determine their spillover and negative impact to the native fish fauna. Furthermore, our results show that tilapias acquired native parasites such as the widespread acantocephalan *N. costarricense* and several trematodes of the genus *Saccocoelioides*.

This study provides for the first-time records of three invasive species of parasites which have successfully spread in the Nicaraguan lakes. As mentioned before, we found the monogenean *C. sclerosus*, but only in the African tilapia, and not yet on a native cichlid. This parasite has spread around the world together with farmed tilapia[61–64]. In addition, we found the Asian tapeworm *Schyzocotyle acheilognathi.* In the Americas, the Asian tapeworm has been so far only reported in two cichlid species in Panama[65], *Aequidens coeruleopunctatus* and *Cryptoheros paramensis*. This parasite was likely introduced into the Panama Canal area with the stocking of grass carps *Ctenopharyngodon idella* for the control of aquatic vegetation. Although no carp introduction has been reported in the Nicaraguan lakes, the Asian tapeworm was already reported in Nicaraguan poeciliids[66]. We also found the cosmopolitan anchorworm *Lernaea cyprinacea*, another parasite species usually co-introduced with carps. To the best of our knowledge, no species carps (Cyprinidae) have been introduced in Nicaraguan lakes. Likewise, other fish species may represent the source of infection, such as poecilids[66–69]. The oribatid mite in the body cavity of *Parachromis* spp. in crater Lake Xiloá may not represent a case of invasive species. Mites are considered unusual fish parasites, although under certain environmental conditions some have been shown to proliferate and colonize weak or stressed fish[70]. Indeed, very few findings of oribatid mites have been reported (but see[71,76]).

Parasites are a useful independent source of information about the evolutionary history of hosts and their diversification patterns. Parasites may act as biological tags of hosts taxonomy and biogeography[52,72,73]. Additional layers of information can be gained by contrasting the genetic structure of parasites and hosts[74,75]. In African cichlids monogenean ectoparasites have been used for evaluating their contribution to the fish species diversity[52,76]. There are interesting open questions in cichlid biogeography, such as the extent of marine dispersal after Gondwanan break-up[77], or the colonization of Central America from South American stocks[78], and parasites might shed additional light[25,52,79].

Over 400 species of parasites have been recorded worldwide from cichlids, and about half of them correspond to American cichlids[25]. This is considered a great underestimation of the real diversity. Increasing work is revealing hidden diversity, and even new genera are being described[80–82]. The incorporation of genetic data to evolutionary parasitology has fueled the recognition of new taxa, revealing cryptic species[83–86], and contributing to the establishment of robust species boundaries, increasing taxonomic resolution. The parasites of freshwater fishes in Central America are still poorly known and many cichlid species remain to be studied.

Comprehensive studies such as the present work provide a solid ground to further explore the evolutionary history of hosts and parasites.

Conclusion

We have compiled the most complete inventory of macroparasites infecting cichlid fish in the Nicaraguan lakes, focusing on the Midas cichlid which represents a model system of recent repeated adaptive radiations in several crater lakes. This study expands further our knowledge of parasite communities in freshwater fish of the Neotropical region, by including a detailed morphological identification in combination with molecular data to fully accomplish species identification. We showed that the great Nicaraguan lakes hold larger parasite diversity than the smaller and younger crater lakes. However, fish populations within the large lakes analysed individually did not have more diverse parasite assemblages in comparison with those of the crater lakes. This study provides the ground for investigating host-parasite dynamics in this promising system, and the contribution of parasites to the species richness of Nicaraguan cichlids.

Ethical approval. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Field study. The Ministry of Natural Resources (MARENA) in Nicaragua provided collection permits (No. 001-012015) to MB.

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Author contributions

MB performed the fish sampling. AS performed the fish dissection and DNA extraction. AS and GPPL identified the parasites. AS performed the analysis. AS, MB and GPPL wrote the manuscript. All authors reviewed and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Figure legends

Figure 1. Photomicrographs of representative species of macroparasites of cichlids from the Nicaraguan lakes. The trematodes: (**a**) *Saccocoleioides* spp., (**b**) *C. cichlasomae,* (**c**) anterior end of *A. compactum*, (**d**) *O. manteri* and (**e**) metacercariae of Cryptogonomidae gen. sp. The monogeneans: (**f**) whole specimen of *S. mexicanum*, (**g**) haptor of *S. mexicanum* and (**h**) haptor of *S. nicaraguense*. The nematodes: (**i**) tail of *Physocephalus* sp., (**j** and **k**) larval stages of *Contracaecum* spp., (**l**) apical view of *P. barlowi* and (**m**) lateral view of the anterior end of *Goezia* sp. The acanthocephalans: (**n**) apical view of the proboscis hooks of the cystacanth *Polymorphus brevis* and (**o**) lateral view of the hooks of *N. costarricense*. The copepods: (**p**) *A. margulisae* anchored to a gill filament, (**q**) Ergasilidae gen. sp. and (**r**) anchor of *L. cyprinacea*. (**s**) Oral sucker of the hirudinean *Myzobdella* sp. (**t**) Ventral view of the branchiuran *Argulus* sp.

Figure 2. Map of the Nicaraguan lakes showing the proportion of parasite taxa per group. Numbers in the pie chart show the total number of parasite taxa per lake.

Figure 3. Matrix of parasite prevalence per host and lake. Dark colors represent increasing prevalence. White cells indicate absences. Host codes are according to Supplementary Table S3. 1) *Contracaecum* spp. and *Hystherothylacium* sp., 2) *S. orosiensis* and *S. cf. lamothei,* and 3) Ergasilidae copepods, were condensed each into a single taxon, and their prevalences were calculated together.

Figure 4. Rarefaction (solid lines) and extrapolation (dotted lines) curves for species richness (order q = 0) with 95% confidence intervals (shaded areas). The

curves indicate the extrapolation of parasite richness according to the number of fish sampled.

Table captions

Table 1. List of the macroparasite species grouped taxonomically found in cichlid fishes from the Nicaraguan lakes. S, stage: adult A, larvae L. T, type of parasite: Ectoparasite Ec, Endoparasite En. Si, site of infection. L, life cycle: direct D, indirect I, unknown ?. * denotes invasive species.

Table 2. Infracommunity diversity indices (Richness, Shannon-Wiener, Simpson) ± 95% confidence intervals for macroparasite taxa for each lake, and p-value of the Kruskall-Wallis or Wilcoxon tests among lakes. Different letters indicate statistically significant differences in paired comparisons (p-values <0.05).

Table 3. Diversity indices for the infracommunities (Mean richness, observed richness, extraplolated richness, Shannon-Wiener and Simpson dominance ± 95% confidence interval). Shaded in gray the values for the Midas cichlids. p-values according to Kruskall-Wallis test. s.e.: standard error.

Supplementary material

Supplementary Table S1. GenBank accession numbers and Colección Nacional de Helmintos (CNHE), Colección Nacional de Crustáceos (CNCR), and Colección Nacional de Ácaros (CNAC) catalog numbers.

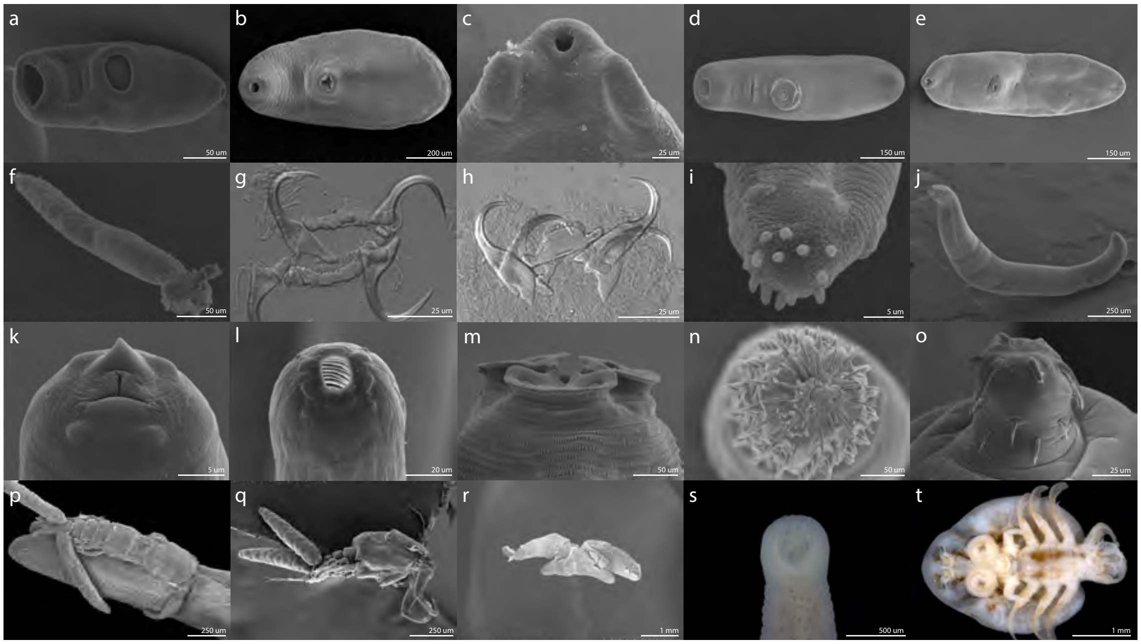
Supplementary Table S2. Primers used for parasite gene amplification. Asterisk indicates primers used only in sequencing reaction.

Supplementary Table S3. Cichlids species collected in the Nicaraguan lakes in the three sampling campaigns, and overall prevalence of macroparasites per year.

Ne, Number of fish examined, Ni, Number of fish infected, P, prevalence (%).

Supplementary Table S4. Records of parasites and their respective hosts within lakes. In bold the Midas cichlids species are indicated.

Figure 1. Photomicrographs of representative species of macroparasites of cichlids from the Nicaraguan lakes. The trematodes: (**a**) *Saccocoleioides* spp., (**b**) *C. cichlasomae,* (**c**) anterior end of *A. compactum*, (**d**) *O. manteri* and (**e**) metacercariae of Cryptogonomidae gen. sp. The monogeneans: (**f**) whole specimen of *S. mexicanum*, (**g**) haptor of *S. mexicanum* and (**h**) haptor of *S. nicaraguense*. The nematodes: (**i**) tail of *Physocephalus* sp., (**j** and **k**) larval stages of *Contracaecum* spp., (**l**) apical view of *P. barlowi* and (**m**) lateral view of the anterior end of *Goezia* sp. The acanthocephalans: (**n**) apical view of the proboscis hooks of the cystacanth *Polymorphus brevis* and (**o**) lateral view of the hooks of *N. costarricense*. The copepods: (**p**) *A. margulisae* anchored to a gill filament, (**q**) Ergasilidae gen. sp. and (**r**) anchor of *L. cyprinacea*. (**s**) Oral sucker of the hirudinean *Myzobdella* sp. (**t**) Ventral view of the branchiuran *Argulus* sp.



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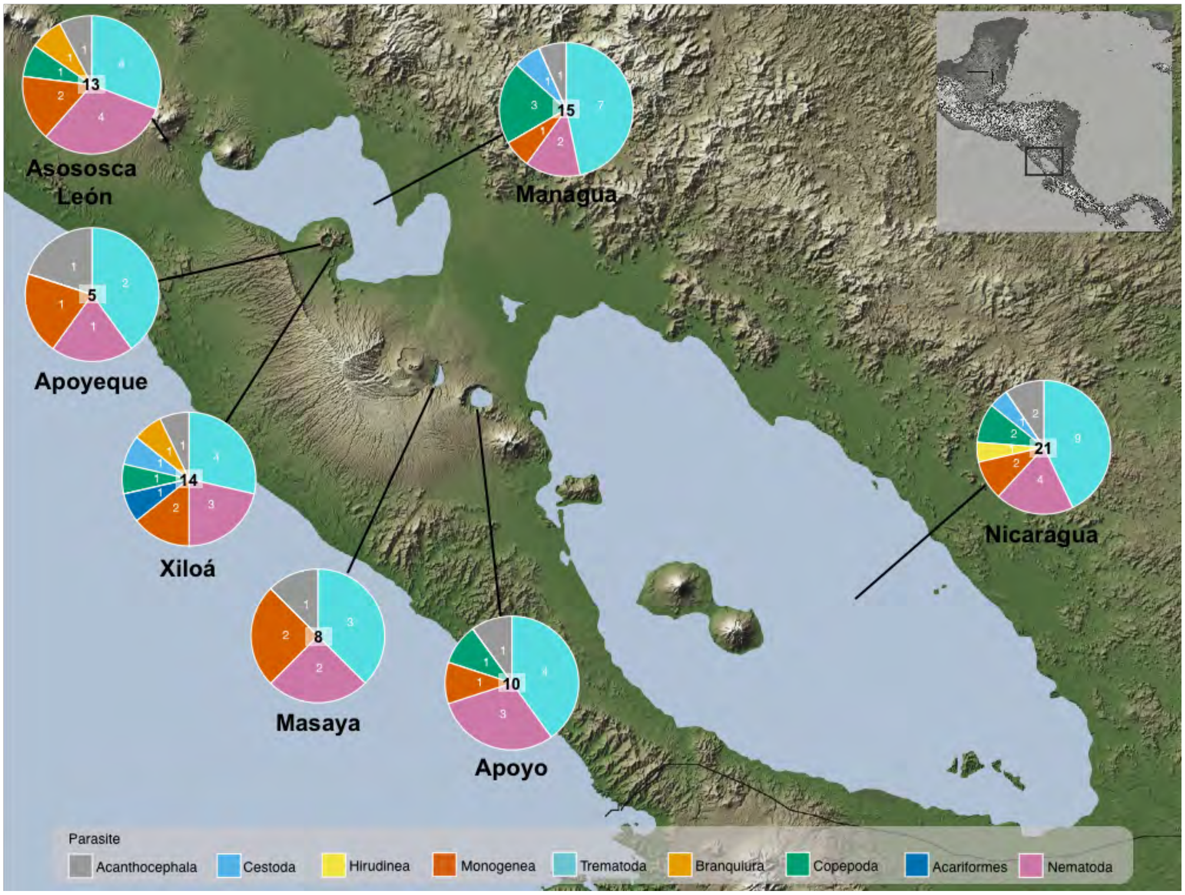

Figure 3. Matrix of parasite prevalence per host and lake. Dark colors represent increasing prevalence. White cells indicate absences. Host codes are according to Supplementary Table S3. 1) *Contracaecum* spp. and *Hystherothylacium* sp., 2) *S. orosiensis* and *S. cf. lamothei,* and 3) Ergasilidae copepods, were condensed each into a single taxon, and their prevalences were calculated together.

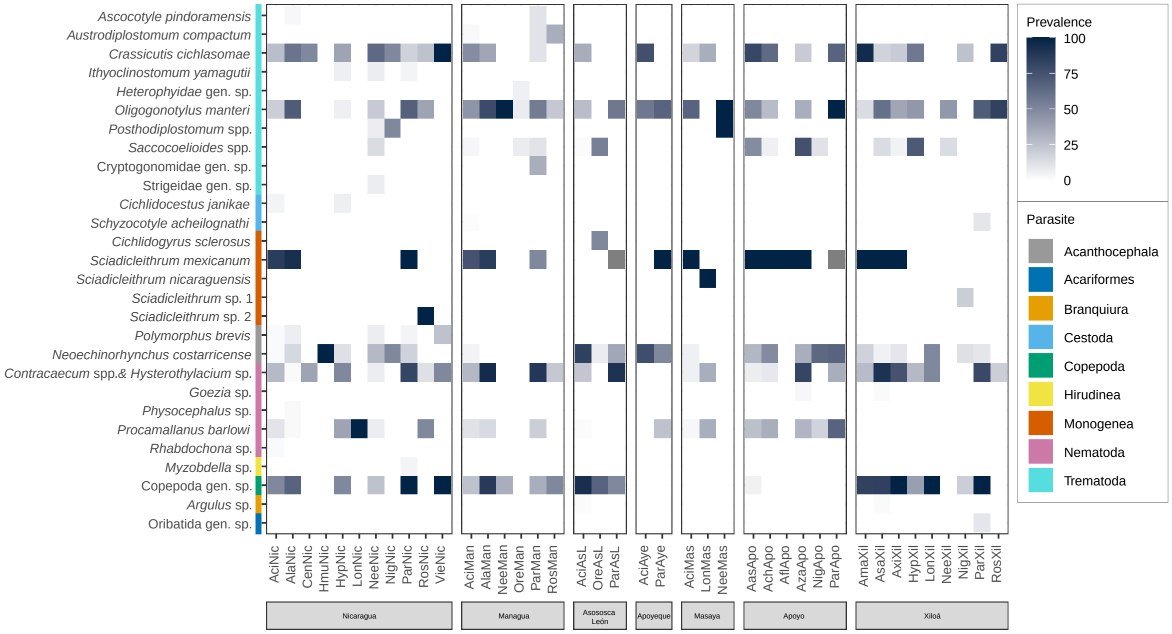

Figure 4. Rarefaction (solid lines) and extrapolation (dotted lines) curves for species richness (order q = 0) with 95% confidence intervals (shaded areas). The curves indicate the extrapolation of parasite richness according to the number of fish sampled.

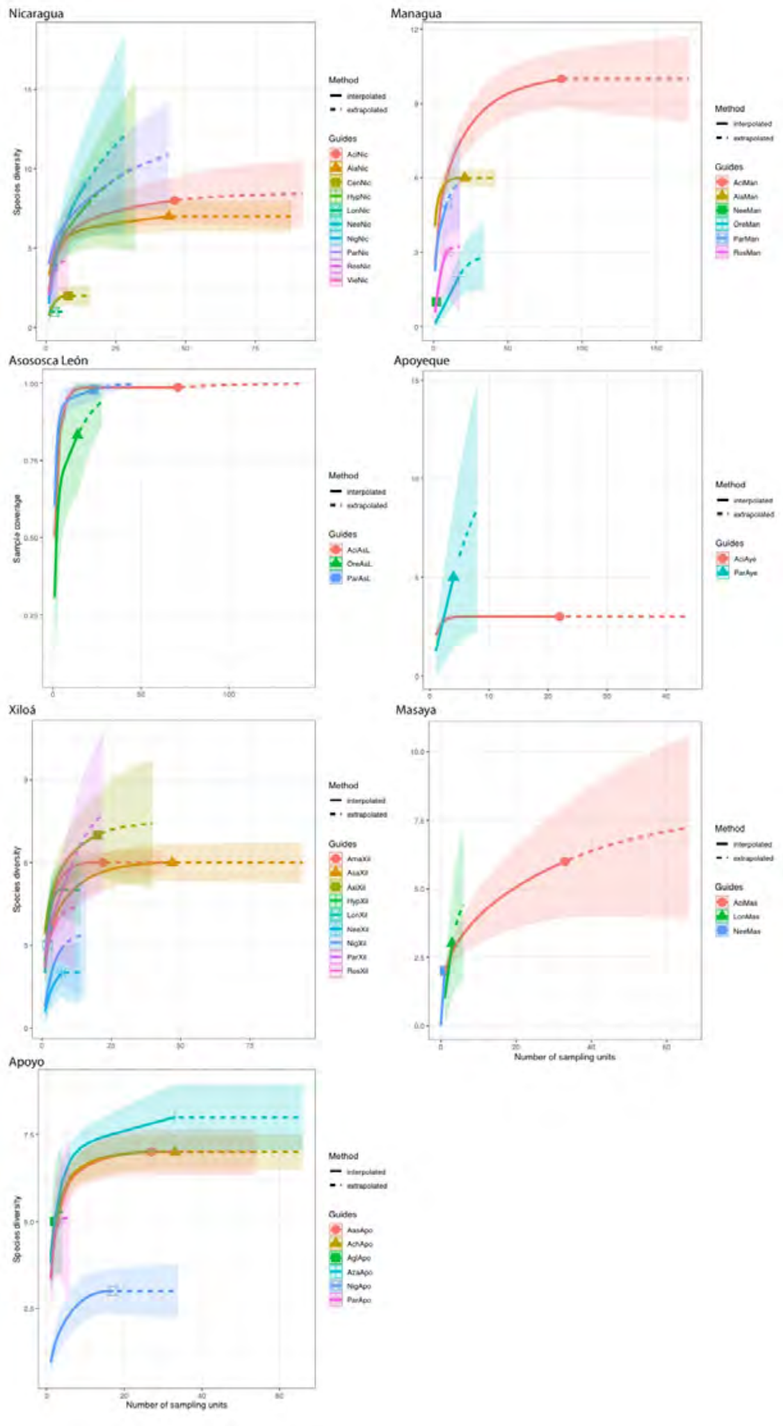

Table 1. List of the macroparasite species grouped taxonomically found in cichlid fishes from the Nicaraguan lakes. S, stage, adult A or larvae L. T, type of parasite, Ectoparasite Ec, or Endoparasite En. Si, site of infection. L, life cycle, direct D, indirect I, or unknown ?. * denote invasive species.

Parasite	**S**	**T**	**Si**	**L**	**Midas cichlids**	**Other-cichlids**
Trematoda (12)						
Ascocotyle pindoramensis	L	Ec	gills	I	✓	✓
Austrodiplostomum compactum	L	Ec	eye	I	✓	✓
Crassicutis cichlasomae	A	En	gut	I	✓	✓
Cryptogonomidae gen. sp.	L	Ec	gills	I	—	✓
Ithyoclinostomum yamagutii	L	En	body cavity	I	—	✓
Heterophyidae gen. sp.	L	En	body cavity	I	—	✓
Oligogonotylus manteri	A	En	gut	I	✓	✓
Posthodiplostomum sp. 1	L	En	body cavity	I	—	✓
Posthodiplostomum sp. 2	L	En	muscle	I	—	✓
Saccocoelioides orosiensis	A	En	gut	I	✓	✓
Saccocoelioides cf. *lamothei*	A	En	gut	I	✓	✓
Strigeidae gen sp.	L	En	body cavity	I	—	✓
Cestoda (2)						
Cichlidocestus janikae	A	En	gut	I	✓	✓
Schyzocotyle acheilognathi *	A	En	gut	I	✓	✓
Monogenea (5)						
Cichlidogyrus sclerosus *	A	Ec	gills	D	—	✓
Sciadicleithrum mexicanum	A	Ec	gills	D	✓	✓
Sciadicleithrum nicaraguense	A	Ec	gills	D	—	✓
Sciadicleithrum sp. 1	A	Ec	gills	D	—	✓
Sciadicleithrum sp. 2	A	Ec	gills	D	—	✓
Acanthocephala (2)						
Polymorphus brevis	L	En	body cavity	I	✓	✓
Neoechinorhynchus costarricense	A	En	gut	I	✓	✓
Nematoda (8)						
Contracaecum sp. 1	L	En	body cavity	I	✓	✓
Contracaecum sp. 2	L	En	body cavity	I	✓	✓
Contracaecum sp. 3	L	En	body cavity	I	✓	✓
Goezia sp.	A	En	gut	I	✓	—
Hysterothylacium sp.	L	En	body cavity	I	—	✓
Physocephalus sp.	L	En	body cavity	I	—	✓
Procamallanus barlowi	A/L	En	gut	I	✓	✓
Rhabdochona sp.	L	En	gut	I	✓	—

Hirudinea (1)						
Myzobdella sp.	A	Ec	skin	D	—	✓
Copepoda (5)						
Acusicola margulisae	A	Ec	gills	D	✓	✓
Acusicola sp.	A	Ec	gills	D	✓	—
Ergasilidae gen. sp.	A	Ec	gills	D	✓	✓
Lernaea cyprinacea	A	Ec	skin	D	—	✓
Lernaeidae gen. sp.	A	Ec	gills	D	✓	✓
Branchiura (1)						
Argulus sp.	A	Ec	skin/mouth	D	✓	—
Acariformes (1)						
Oribatida gen. sp.	A	En	body cavity	?	—	✓

Table 2. Infracommunity diversity indices (Richness, Shannon-Wiener, Simpson; average+/- 95% confidence interval) for macroparasite taxa among lakes; p-values <0.05 indicate significative differences among lakes based on Kruskall-Wallis or Wilcoxon tests. Different letters indicate statistically significant differences in paired comparisons (p-values <0.05).

	Great lakes		Crater lakes					
Index	Nicaragua^{a}	Managua^{b}	Asososca León^{c}	Apoyeque^{d}	Xiloá^{e}	Masaya^{f}	Apoyo^{g}	p-value
Species richness	2.45 ± 0.38 ^{g}	2.21 ± 0.43 ^{g}	2.82 ± 0.41 ^{dg}	2.09 ± 0.41 ^{cg}	2.77 ± 0.35 ^{g}	2.47 ± 0.59 ^{g}	3.53 ± 0.39 ^{abcdef}	**p < 0.0001**
Shannon-Wiener	0.27 ± 0.05 ^{g}	0.27 ± 0.05 ^{g}	0.23 ± 0.06 ^{g}	0.38 ± 0.15 ^{fe}	0.14 ± 0.16 ^{dg}	0.13 ± 0.12 ^{dg}	0.41 ± 0.06 ^{abce}	**p < 0.0001**
Simpson	0.36 ± 0.05	0.39 ± 0.06 ^{ce}	0.24 ± 0.07 ^{b}	0.22 ± 0.09	0.21 ± 0.07 ^{b}	0.36 ± 0.12	0.29 ± 0.06	**p < 0.0001**

Table 3. Diversity indices for the infracommunities (Mean richness, observed richness, extraplolated richness, Shannon-Wiener and Simpson dominance ± 95% confidence interval). Shaded in gray the values for the Midas cichlids. p-values according to Kruskall-Wallis test. s.e.: standard error.

Host-lake combination	Mean richness	Observed richness	Extrapolated richness and estimated bootstrap s.e.	Shannon-Wiener	Simpson
Nicaragua					
Amphilophus citrinellus	2.50 ± 1.00	8	8.48 ± 1.29	0.12 ± 0.10	0.36 ± 0.11
Amphilophus labiatus	3.47 ± 0.72	7	7.00 ± 0.48	0.37 ± 0.10	0.36 ± 0.11
Amatitlania nigrofasciata	2.00 ± 2.00	3	4.50 ± 2.25	0.34 ± 0.41	0.25 ± 0.55
Archocentrus centrarchus	1.00 ± 0.67	2	2.00 ± 0.34	0.14 ± 0.22	0.47 ± 0.27
Cribroheros longimanus	1	1	1.00 ± 0.20	0	0
Cribroheros rostratus	3	3	4.00 ± 2.00	0.17 ± 0.21	0.21 ± 0.23
Hypsophrys nematopus	2.25 ± 1.05	9	16.42 ± 10.85	0.30 ± 0.17	0.25 ± 0.20
Hypsophrys nicaraguensis	1.87 ± 0.81	8	12.21 ± 6.76	0.40 ± 0.16	0.55 ± 0.18
Parachromis spp.	3.66 ± 1.84	9	11.86 ± 4.28	0.41 ± 0.14	0.35 ± 0.15
Vieja sp.	4	4	4.18 ± 0.58	0.21 ± 0.36	0.64 ± 0.32
p-value	**$p < 0.01$**			**$p < 0.05$**	0.191
Managua					
Amphilophus citrinellus	2.44 ± 0.69	10	10.00 ± 0.58	0.26 ± 0.07	0.30 ± 0.07
Amphilophus labiatus	4.20 ± 0.91	6	6.00 ± 0.21	0.63 ± 0.15	0.40 ± 0.16
Cribroheros rostratus	1.00 ± 1.03	3	3.22 ± 0.66	0.07 ± 0.24	0.72 ± 0.23
Hypsophrys nematopus	1	1	1.00 ± 0.28	0	0
Oreochromis sp.	0.50 ± 0.66	2	2.94 ± 1.92	0	0.84 ± 0.20
Parachromis spp.	2.00 ± 1.95	5	5.88 ± 1.97	0.25 ± 0.23	0.25 ± 0.23
p-value	**$p < 0.0001$**			**$p < 0.05$**	0.310
Asososca León					
Amphilophus citrinellus	3.00 ± 0.55	7	7.98 ± 2.20	0.29 ± 0.08	0.22 ± 0.09
Oreochromis sp.	2.33 ± 1.51	4	5.85 ± 3.49	0	0.38 ± 0.19
Parachromis spp.	2.28 ± 1.02	5	5.47 ± 1.27	0.18 ± 0.15	0.19 ± 0.15
p-value	0.227			0.085	0.085
Apoyeque					
Amphilophus citrinellus	2.09 ± 0.42	3	3	0.39 ± 0.16	0.22 ± 0.10
Parachromis spp.		5	12.50 ± 7.57	0.34 ± 0.39	0.23 ± 0.28
p-value	0.864			0.860	0.860
Xiloá					
Amphilophus amarillo	2.92 ± 0.73	6	6.00 ± 0.25	0.21 ± 0.15	0.18 ± 0.16
Amphilophus sagittae	3.60 ± 0.62	6	6.00 ± 0.35	0.04 ± 0.11	0.08 ± 0.12
Amphilophus xiloaensis	3.11 ± 0.90	7	7.47 ± 1.26	0.11 ± 0.15	0.16 ± 0.16
Amatitlania nigrofasciata	1.12 ± 1.14	3	3.43 ± 1.18	0	0.62 ± 0.27

Cribroheros longimanus	4	3	4.00 ± 2.00	0.23 ± 0.30	0.12 ± 0.49
Cribroheros rostratus	2.50 ± 1.32	4	4.41 ± 1.13	0.45 ± 0.29	0.31 ± 0.34
Hypsophrys nematopus	0.75 ± 2.20	2	2.00 ± 0.49	0.09 ± 0.26	0.64 ± 0.28
Hypsophrys nicaraguensis	2.20 ± 1.47	5	5.00 ± 0.46	0.46 ± 0.24	0.29 ± 0.10
Parachromis spp.	5.50 ± 0.97	6	8.72 ± 4.03	0.07 ± 0.22	0.03 ± 0.22
p-value	**p < 0.001**			0.1248	**p < 0.01**
Masaya					
Amphilophus citrinellus	2.58 ± 0.65	6	7.93 ± 3.63	0.11 ± 0.13	0.29 ± 0.14
Cribroheros longimanus	3	3	5.00 ± 2.92	0.23 ± 0.43	0.50 ± 0.46
Hypsophrys nematopus	3	2	2	0.69	0.5
p-value	0.128			0.180	0.200
Apoyo					
Amphilophus astorquii	3.63 ± 0.68	7	7.00 ± 0.34	0.55 ± 0.12	0.33 ± 0.14
Amphilophus chancho	3.37 ± 0.69	7	7.00 ± 0.21	0.44 ± 0.12	0.37 ± 0.12
Amphilophus zaliosus	4.76 ± 0.77	8	8.00 ± 0.47	0.40 ± 0.11	0.24 ± 0.13
Amphilophus globosus	4.00 ± 1.95	5	5.33 ± 0.77	0.39 ± 0.14	0.19 ± 0.08
Amatitlania nigrofasciata	1.28 ± 1.17	3	3.00 ± 0.39	0.04 ± 0.16	0.14 ± 0.18
Parachromis spp.	3.33 ± 1.77	5	5.11 ± 0.40	0.95 ± 0.40	0.55 ± 0.41
p-value	**p < 0.001**			**p < 0.001**	**p < 0.01**

Supplementary Table S1. GenBank accession numbers and CNHE, CNCR or CNAC catalog numbers.

Parasite	cox1	cox2	28S	18S	CNHE	CNCR	CNAC
Trematoda							
Austrodiplostomum compactum	−	−	XXXXX	−	11496	−	−
Crassicutis cichlasomae	XXXXX	−	XXXXX	−	11483−11486	−	−
Ithyoclinostomum yamagutii	−	−	XXXXX	−	11498	−	−
Heterophyidae gen. sp.	−	−	−	−	11504	−	−
Oligogonotylus manteri	XXXXX	−	XXXXX	−	11487−11490	−	−
Posthodiplostomum sp. 1	−	−	−	−	11500	−	−
Posthodiplostomum sp. 2	−	−	−	−	11501	−	−
Saccocoelioides orosiensis	XXXXX	−	−	−	−	−	−
Saccocoelioides cf. *lamothei*	XXXXX	−	−	−	−	−	−
Monogenea							
Cichlidogyrus sclerosus	XXXXX	−	−	−	−	−	−
Sciadicleithrum mexicanum	XXXXX	−	XXXXX	−	11475−11480	−	−
Sciadicleithrum nicaraguense	XXXXX	−	XXXXX	−	11481	−	−
Sciadicleithrum sp. 1	XXXXX	−	XXXXX	−	−	−	−
Sciadicleithrum sp. 2	XXXXX	−	−	−	−	−	−
Acanthocephala							
Neoechinorhynchus costarricense	XXXXX	−	XXXXX	−	11507	−	−
Nematoda							
Contracaecum sp. 1	−	XXXXX	−	−	−	−	−
Contracaecum sp. 2	−	XXXXX	−	−	−	−	−
Contracaecum sp. 3	−	XXXXX	−	−	−	−	−
Goezia sp.	−	XXXXX	−	−	−	−	−
Hysterothylacium sp.	−	−	−	−	11506	−	−
Physocephalus sp.	−	−	−	−	11505	−	−
Procamallanus barlowi	XXXXX	−	XXXXX	−	11472−11473	−	−
Hirudinea							
Myzobdella sp.	XXXXX	−	−	−	−	−	−

Copepoda						–	–
Acusicola margulisae	XXXXX	–	XXXXX	XXXXX	–	35553−35553	–
Acusicola sp.	–	–	–	–	–	–	–
Ergasilidae gen. sp.	XXXXX	–	–	–	–	–	–
Lernaeidae gen. sp.	–	–	–	–	–	–	–
Acariformes							
Oribatide gen. sp.	–	–	–	–		–	XXXXX

Supplementary Table S2. Primers used for PCR and sequencing of parasites in this study.

Genetic region	Primer name	F/ R	Sequence (5'− 3')	Reference
cox1	JB3/ Asmit1	F	TTTTTTGGGCATCCTGAGGTTTAT	1
	JB4.5	R	TAAAGAAAGAACATAATGAAAATG	1
	Schisto3	R	TAATGCATMGGAAAAAAACA	2
	507	F	AGTTCTAATCATAARGATATYGG	3
	HCO2198	R	TAAACTTCAGGGTGACCAAAAAATCA	4
	pr-b	R	AGTTCTAATCATAARGATATYGG	5
	MplatCOX1dF	F	TGTAAAACGACGGCCAGTTTWCITTRGATCATAAG	6
	MplatCOX1dR	R	CAGGAAACAGCTATGACTGAAAYAAYAIIGGATCICCACC	6
cox2	210	F	TTTTCTAGTTATATAGATTGRTTYAT	7
	211	R	CACCAACTCTTAAAATTATC	7
28S	391	F	AGCGGAGGAAAAGAAACTAA	8
	536	R	CAGCTATCCTGAGGGAAAC	9
	502*	F	CAAGTACCGTGAGGGAAAGTTGC	9
	28SF	F	ACAACTGTGATGCCCTTAG	10
	28SR	R	TGGTCCGTGTTTCAAGACG	10
	U178	F	GCACCCGCTGAAYTTAAG	2
	1200R*	R	GCATAGTTCACCATCTTTCGG	2
	L1642	R	CCAGCGCCATCCATTTTCA	2
18S	18SF	F	AAGGTGTGMCCTATCAACT	10
	18SR	R	TTACTTCCTCTAAACGCTC	10

F: forward primer; R: reverse primer

Asterisk indicates primers used only in sequencing reaction.

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Supplementary Table S3. Species of cichlids sampled in two great and five crater lakes of Nicaragua between 2017 and 2019, indicating overall prevalence of macroparasites per sampling year. Ne. Number of fish examined, Ni. Number of fish infected, P. prevalence (%).

Lake	Host species	Host code	Year									
			2017			2018			2019			
			Ne	Ni	P	Ne	Ni	P	Ne	Ni	P	Total
Asososca León (108)	***Amphilophus citrinellus***	AciAsL	20	20	100	28	28	100	23	23	100	71
	Oreochromis sp.	OreAsL	3	3	100	3	3	100	8	3		14
	Parachromis spp.	ParAsL	5	5	100	12	12	100	6	6	100	23
Apoyeque (26)	***Amphilophus citrinellus***	AciAye	22	22	100	–	–	–	–	–	–	22
	Parachromis spp.	ParAye	4	4	100	–	–	–	–	–	–	4
Apoyo (117)	***Amphilophus astorquii***	AasApo	16	16	100	4	4	100	7	7	100	27
	Amphilophus chancho	AchApo	15	15	100	5	5	100	13	13	100	33
	Amphilophus flavealus	AflApo	1	1	100	–	–	–	–	–	–	1
	Amphilophus zaliosus	AzaApo	14	14	100	5	5	100	14	14	100	33
	Amphilophus globosus	AglApo	–	–	–	–	–	–	3	3	100	3
	Amatitlania nigrofasciata	NigApo	–	–	–	11	10	90.9	6	5		17
	Parachromis spp.	ParApo	–	–	–	3	3	100	–	–		3
Masaya (39)	***Amphilophus citrinellus***	AciMas	25	25	100	10	10	100	–	–	–	35
	Cribroheros longimanus	LonMas	3	3	100	–	–	–	–	–	–	3
	Hypsophrys nematopus	NeeMas	–	–	–	1	1	100	–	–	–	1
Xiloá (145)	***Amphilophus amarillo***	AmaXil	16	16	100	5	3	60	1	1	100	22
	Amphilophus sagittae	AsaXil	16	16	100	27	27	100	6	6	100	49
	Amphilophus xiloaensis	AxiXil	11	11	100	4	4	100	7	7	100	22
	Amatitlania nigrofasciata	NigXil	–	–	–	5	3		4	4	100	9
	Cribroheros longimanus	LonAxil	–	–	–	2	2	100	3			5
	Cribroheros rostratus	RosXil	–	–	–	3	3	100	4	4	100	7
	Hypsophrys nematopus	NeeXil	–	–	–	4	2	50	3	2		7
	Hypsophrys nicaraguensis	HypXil	–	–	–	7	7	100	6			13
	Parachromis spp.	ParXil	5	5	100	5	5	100	1	1	100	11
Managua (152)	***Amphilophus citrinellus***	AciMan	30	29	96.6	30	30	100	26	26	100	86
	Amphilophus labiatus	AlaMan	9	9	100	7	7	100	5	5	100	21
	Cribroheros rostratus	RosMan	1	1	100	3	3	100	6	5		10
	Hypsophrys nematopus	NeeMan	–	–	–	–	–	–	8		100	8
	Oreochromis sp.	OreMan	–	–	–	6	0	0	11	7		17
	Parachromis spp.	ParMan	3	3	100	4	4	100	3	3	100	10
Nicaragua (167)	***Amphilophus citrinellus***	AciNic	9	9	100	21	21	100	15	15	100	45
	Amphilophus labiatus	AlaNic	14	14	100	13	13	100	12	12	100	39
	Amatitlania nigrofasciata	NigNic	–	–	–	–	–	–	3		100	3
	Archocentrus centrarchus	CenNic	–	–	–	–	–	–	8	7		8
	Cribroheros longimanus	LonNic	–	–	–	1	1	100	2	2	100	3
	Cribroheros rostratus	RosNic	–	–	–	8	8	100	3		100	11
	Herotilapia multispinosa	HmuNic	–	–	–	–	–	–	1	1	100	1
	Hypsophrys nematopus	NeeNic	–	–	–	13	13	100	1	1	100	14
	Hypsophrys nicaraguensis	HypNic	–	–	–	13	12		3	2		16
	Parachromis spp.	ParNic	3	3	100	6	6	100	14	14	100	23
	Vieja sp.	VieNic	1	0	0	3	3	100	–	–	–	4

Supplementary Table 4. Host-parasite records within lakes. In bold the Midas cichlids species are indicated.

Lake	Species	Host species
Trematoda		
Nicaragua	*Ascocotyle pindoramensis*	***A. labiatus***
	Crassicutis cichlasomae	***A. citrinellus, A. labiatus***, *A. centrarchus, A. nigrofasciata, C. rostratus, H. nicaraguensis, H. nematopus, Parachromis* spp., *Vieja* sp.
	Ithyoclinostomum yamagutii	*H. nicaraguensis, H. nematopus, Parachromis* spp.
	Oligogonotylus manteri	***A. citrinellus, A. labiatus***, *C. rostratus, H. nicaraguensis, H. nematopus, Parachromis* spp.
	Posthodiplostomum sp. 1	*H. nematopus*
	Posthodiplostomum sp. 2	*A. nigrofasciata*
	Saccocoelioides cf. *lamothei*	*H. nematopus*
	Saccocoleioides spp.	*H. nematopus*
	Strigeidae gen sp.	*H. nematopus*
Managua	*Ascocotyle pindoramensis*	*Parachromis sp.*
	Austrodiplostomum compactum	***A.citrinellus***, *C. rostratus, Parachromis* spp.
	Crassicutis cichlasomae	***A. citrinellus, A. labiatus***, *C. rostratus, Parachromis* spp.
	Cryptogonomidae gen. sp.	*Parachromis spp.*
	Heterophyidae gen. sp.	*Oreochromis* sp.
	Oligogonotylus manteri	***A. citrinellus, A. labiatus***, *C. rostratus, H. nematopus, Parachromis* spp.
	Saccocoleioides spp.	***A. citrinellus***, *Oreochromis* sp., *Parachromis* spp.
Asososca León	*Crassicutis cichlasomae*	***A. citrinellus***
	Oligogonotylus manteri	***A. citrinellus,*** *Parachromis* spp.
	Saccocoelioides cf. *lamothei*	***A. citrinellus***, *Oreochromis* sp.
	Saccocoelioides orosiensis	*Oreochromis* sp.
	Saccocoleioides spp.	***A. citrinellus***, *Oreochromis* sp.
Apoyeque	*Crassicutis cichlasomae*	***A. citrinellus***, *Parachromis* spp.
	Oligogonotylus manteri	***A. citrinellus***, *Parachromis* spp.
Xiloá	*Crassicutis cichlasomae*	***A. amarillo, A. sagittae, A. xiloaensis***, *C. longimanus, A. nigrofasciata, C. rostratus, H. nicaraguensis*
	Oligogonotylus manteri	***A. amarillo, A. sagittae, A. xiloaensis***, *C. longimanus, C. rostratus, H. nicaraguensis, H. nematopus, Parachromis* spp.
	Saccocoelioides orosiensis	***A. sagittae, A. xiloaensis***, *H. nicaraguensis, H. nematopus*
	Saccocoleioides spp.	***A. sagittae***
Masaya	*Crassicutis cichlasomae*	***A. citrinellus***, *C. longimanus*
	Oligogonotylus manteri	***A. citrinellus***, *H. nematopus*
	Posthodiplostomum sp. 1	*H. nematopus*
Apoyo	*Crassicutis cichlasomae*	***A. astorquii, A. chancho, A. globosus, A. flaveolus, A. zaliosus***, *Parachromis* spp.
	Oligogonotylus manteri	***A. astorquii, A. chancho, A. globosus, A. zaliosus***, *Parachromis spp.*

	Saccocoelioides orosiensis	***A. astorquii, A. chancho, A. zaliosus***, *A. nigrofasciata*
	Saccocoleioides spp.	***A. astorquii, A. chancho, A. flaveolus, A. globosus, A. zaliosus***
Cestoda		
Nicaragua	*Cichlidocestus janikae*	***A. citrinellus***, *H. nicaraguensis*
Managua	*Schyzocotyle acheilognathi*	***A. citrinellus***
Xiloá	*Schyzocotyle acheilognathi*	*Parachromis* spp.
Monogenea		
Nicaragua	*Sciadicleithrum mexicanum*	***A. citrinellus***, *Parachromis* spp.
	Sciadicleithrum sp. 2	*C. rostratus*
Managua	*Sciadicleithrum mexicanum*	***A. citrinellus***, *Parachromis* spp.
Asososca León	*Cichlidogyrus sclerosus*	*Oreochromis* sp.
	Sciadicleithrum mexicanum	*Parachromis* spp.
Apoyeque	*Sciadicleithrum mexicanum*	*Parachromis* spp.
Xiloá	*Sciadicleithrum mexicanum*	***A. amarillo, A. sagittae, A. xiloaensis***
	Sciadicleithrum sp. 1	*A. nigrofasciata*
Masaya	*Sciadicleithrum mexicanum*	***A. citrinellus***
	Sciadicleithrum nicaraguensis	*C. longimanus*
Apoyo	*Sciadicleithrum mexicanum*	***A. astorquii, A. flaveolus, A. chancho, A. zaliosus***, *Parachromis* spp.
Acantocephala		
Nicaragua	*Polymorphus brevis*	***A. citrinellus, A. labiatus***, *H. nematopus, Parachomis spp.,Vieja* sp.
	Neoechinorynchus costarricense	***A. labiatus***, *A. nigrofasciata, H. nematopus, H. nicaraguensis, H. multispinosa, Parachromis* spp.
Managua	*Neoechinorynchus costarricense*	***A. citrinellus***
Asososca León	*Neoechinorynchus costarricense*	***A. citrinellus***, *Parachromis* spp., *Oreochromis* sp.
Apoyeque	*Neoechinorynchus costarricense*	***A. citrinellus***, *Parachromis* spp.
Xiloá	*Neoechinorynchus costarricense*	***A. amarillo, A. xiloaensis, A. sagittae,*** *A. nigrofasciata, C. longimanus, Parachromis* spp.
Masaya	*Neoechinorynchus costarricense*	***A. citrinellus***
Apoyo	*Neoechinorynchus costarricense*	***A. astorquii, A. chancho, A. zaliosus***, *A. nigrofasciata, Parachromis* spp.
Nematoda		
Nicaragua	*Contracaecum* sp. 1	***A. citrinellus, A. labiatus***, *A. centrarchus, C. rostratus, H. nicaraguensis, H. nematopus, Parachromis* spp., *Vieja* sp.
	Physocephalus sp.	***A. labiatus***
	Procamallanus barlowi	***A. citrinellus***, ***A. labiatus***, *C. longimanus, C. rostratus, H. nicaraguensis, H. nematopus*
	Rhabdochona sp.	***A. citrinellus***
Managua	*Contracaecum* sp. 1	***A. citrinellus, A. labiatus***, *C. rostratus, Parachromis* spp.
	Procamallanus barlowi	***A. citrinellus, A. labiatus***, *Parachromis* spp.,
Asososca León	*Contracaecum* sp. 1	***A. citrinellus***, *Parachromis* spp.
	Contracaecum sp. 2	***A. citrinellus***, *Parachromis* spp.

	Contracaecum sp. 3	***A. citrinellus***, *Parachromis* spp.
	Procamallanus barlowi	***A. citrinellus***
Apoyeque	*Procamallanus barlowi*	*Parachromis* spp.
Xiloá	*Contracaecum* sp. 1	***A. amarillo, A. sagittae, A. xiloaensis***, *C. rostratus, H. nicaraguensis, H. nematopus, Parachromis* spp.
	Goezia sp.	***A. sagittae***
	Hysterothylacium sp.	*Parachromis* spp.
Masaya	*Contracaecum* sp. 1	***A. citrinellus***, *C. longimanus*
	Procamallanus barlowi	***A. citrinellus***, *C. longimanus*
Apoyo	*Contracaecum* sp. 1	***A. astorquii, A. chancho, A. zaliosus,*** *Parachromis* spp.
	Goezia sp.	***A. zaliosus***
	Procamallanus barlowi	***A. astorquii, A. chancho, A. zaliosus***, *A. nigrofasciata, Parachromis* spp.
Hirudinea		
Nicaragua	*Myzobdella* sp.	*Parachromis* spp.
Copepoda		
Nicaragua	*Acusicola* sp.	***A. citrinellus, A. labiatus***
	Ergasilidae gen. sp.	***A. citrinellus, A. labiatus***, *H. nematopus, H. nicaraguensis, Parachromis* spp., *Vieja* sp.
Managua	Ergasilidae gen. sp.	***A. citrinellus, A. labiatus***, *C. rostratus, Parachromis* spp.
	Lernaea cyprinacea	*H. nematopus*
	Lernaeidae gen. sp.	***A. citrinellus, A. labiatus***
Asososca León	*Acusicola margulisae*	***A. citrinellus***, *Parachromis* spp., *Oreochromis* sp.
Xiloá	Ergasilidae gen. sp.	***A. amarillo, A. sagittae, A. xiloaensis,*** *A. nigrofasciata, C. longimanus, H. nicaraguensis, Parachromis* spp.
Apoyo	Ergasilidae gen. sp.	***A. astorquii***
Branchiura		
Asososca León	*Argulus* sp.	***A. citrinellus***
Xiloá	*Argulus* sp.	***A. sagittae***
Acariformes		
Xiloá	Oribatide gen. sp.	*Parachromis* spp.

Filling the knowledge gap of Middle American freshwater fish parasite biodiversity: Metazoan parasite fauna of Nicaragua

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Abstract

The heterogeneous landscape of Nicaragua harbors a large diversity of freshwater fishes. The great Nicaraguan lakes, Managua and Nicaragua, and several adjacent crater lakes, harbor numerous endemic fish species. However, the information about their parasite fauna is still fragmentary. Here, we present the results of a parasite survey focused on the great Nicaraguan lakes and four crater lakes. We provide new data for 17 metazoan parasite taxa infecting seven fish host species. We also gathered all the published records from the literature of the diversity of parasites reported so far from Nicaraguan freshwater fishes. With this information we built a parasite-host and a host-parasite checklist. With data from near 50% of the native and endemic freshwater fishes in Nicaragua, the parasite fauna comprises 101 taxa in 51 fish species allocated in 11 families. Cichlids are the most diverse group of fishes in this region and were most extensively surveyed for their metazoan parasites. Helminths are the best-represented groups of metazoan parasites, with 42 trematodes, five cestodes, 24 monogeneans, two acanthocephalans, 20 nematodes, and one hirudinean. Additionally, freshwater fishes are parasitized by copepods, branchiurans and oribatid mites. Even though the inventory is not yet complete, the patterns of diversity uncovered thus far revealed promising information about the origin, biogeography, and evolutionary history of the Nicaraguan freshwater fish parasite fauna. More studies are necessary to complete our knowledge about the diversity, host association, and distribution of metazoan parasites in Nicaragua and other Central American countries.

Key words: diversity, crater lakes, helminths, inventory.

Introduction

The geographical position of Nicaragua in Lower Central America, along with Costa Rica and Panama, places the region in a pivotal biogeographical position bridging North and South America. The uplift of the Isthmus of Panama created a land bridge which allowed the dispersal of freshwater fauna through the biogeographical event known as the Great American Biotic Interchange (GABI) (see Bacon *et al.*, 2016 and references therein). Irrespective of the date of the closure of the seaway, freshwater fishes dispersed northwards throughout different routes, and experienced diversification events (Perdices *et al.*, 2002; Hulsey *et al.*, 2004; Ornelas-García *et al.*, 2008; Říčan *et al.*, 2016). Nicaragua lies on one of the most active volcanic areas on the planet, the Central American volcanic arc. Over this arch, on the Pacific coast, lie the great Nicaraguan lakes, Managua and Nicaragua. These lakes were formed in the early Pleistocene, and were originally connected, separating later when the lakes opened towards the Atlantic Ocean via the San Juan River (Villa, 1976). In the surrounding of the great lakes lies a chain of active volcanoes dating since the Holocene (Kutterolf *et al.*, 2007). The calderas of the volcanoes collapsed and filled with water from precipitation and/or underground seeps, creating lakes of different ages and isolated environments without interconnections with other waterbodies. Apoyo is the largest caldera and one of the oldest (Kutterolf *et al.*, 2007). Other ancient lakes have experienced recent eruptions and are still active, such as crater Lake Apoyeque which formed ca.17,000 years ago (Avellán *et al.*, 2014). More recent lakes such as the Masaya caldera formed 6,000 years ago was active until 1,800 years ago (Kutterolf *et al.*, 2007). Besides the lakes, Nicaragua has dozens of rivers, several draining from the Central Highlands towards the Atlantic Lowlands.

The heterogenous landscape of this region has shaped the evolution of its biodiversity, with 114 species of fish, of which 104 are native, belonging to 28 families, (Froese & Pauly, 2021). The most diverse freshwater fish group is the Family Cichlidae (28 species), followed by Poeciliidae (10 species) and Characidae (seven species) (Froese & Pauly, 2021). Within cichlids, the Midas cichlid, *Amphilophus* spp., forms the largest biomass in the lakes, and experienced adaptive radiations in several crater lakes (Barluenga *et al.*, 2006; Kautt *et al.*, 2020). These three fish families have south

American origin, and expanded northwards in their dispersal routes (Ornelas-García *et al.*, 2008; Říčan *et al.*, 2008, 2013, 2016; Reznick *et al.*, 2017; García-Andrade *et al.*, 2021).

The knowledge on the parasite diversity of freshwater fishes in this region is still scarce, representing a gap in the parasitological knowledge of the parasite fauna between South and North America, where a larger number of studies have been conducted. In Nicaragua, the first study was performed by Watson (1976) where several species of digeneans from Lake Nicaragua were reported. After that, few studies were conducted across Nicaragua, representing isolated records of parasites in some fish species in scattered sampling areas in Nicaragua (Aguirre-Macedo *et al.*, 2001b; a; Vidal-Martínez *et al.*, 2001b; Mendoza-Franco *et al.*, 2003; González-Solís & Moravec, 2004; Scholz *et al.*, 2004; Andrade-Gómez *et al.*, 2017; López-Jiménez *et al.*, 2018; González-García *et al.*, 2021). Particularly for the crater lakes, McCrary *et al.* (2001) referred an outbreak of blindness among native cichlids in crater Lake Apoyo caused by a trematode infection. Also, some nematodes were reported from crater lakes Xiloá and Apoyo (González-Solís & Jiménez-García, 2006). Recently, intensive survey work aimed at describing the metazoan parasite fauna of cichlids, with an emphasis on the Midas cichlid species complex, yielded the description of an additional set of species (Santacruz *et al.*, 2020, 2021a, 2021b).

The main objective of this paper was first, to characterize the parasites infecting non-cichlid fishes in the two great Nicaraguan lakes and four crater lakes based on field surveys, and second, to provide an overview of all the records published of parasites infecting fishes dwelling the Nicaraguan freshwater systems to briefly discuss their historical biogeography.

Materials and methods

Sampling and fish dissection

Fish were sampled in three consecutive years (2017-2019), at the end of the rainy season (November and December), in the two great lakes Nicaragua and Managua and in four crater lakes (Asososca León, Xiloá, Masaya and Apoyo). The procedures to sample, maintain and euthanize the fish species follow those described in Santacruz *et* *al.* (2021a) and follow procedures accepted by the American Veterinary Medical Association (Leary & Underwood, 2020). All parasites recovered were counted and representative samples of each morphotype were fixed for morphological or molecular study in hot formalin or molecular grade ethanol, respectively.

Parasite processing and identification

All parasites collected were analyzed morphologically to achieve the highest taxonomic resolution following Santacruz *et al.* (2021a). Morphological vouchers, and in some cases hologenophores (*sensu* Pleijel *et al*. 2008), were deposited at the Colección Nacional de Helmintos (CNHE), UNAM, Mexico City: 11482, 11491, 11492, 11497, 11499, 11500 and 11503. In some cases, parasite specimens representing particular morphotypes were sequenced for identification, particularly those whose morphological characteristics or developmental stage impeded reaching an identification to the lowest taxonomic range. The molecular markers used were the mitochondrial cox1, cox2, and the nuclear 18S genes, depending on the genetic library available for each parasite group. The description of the methods for sequencing and primers used can be seen at Santacruz *et al.* (2020, 2021a, 2021b). Sequence data was deposited in GenBank under accession numbers: XXXX–XXXX.

All existing records of parasites from freshwater fishes were retrieved from the literature, considering all published accounts for the period 1976 – 2021. Host names follow those currently recognized in FishBase (Froese & Pauly, 2021). The checklist of the freshwater fishes of Nicaragua was retrieved from the same source.

Results

We analyzed 136 fishes from seven species (see table 1) during the three sampling seasons. The analysis yielded 17 parasite taxa, including trematodes, cestodes, monogeneans, nematodes, copepods and oribatid mites (table 1). Specimens of *Poecilia* spp. were collected in all lakes. Overall, poeciliids were infected by six parasite taxa, including trematodes, cestodes, copepods and oribatid mites. In the crater lakes Asososca León and Xiloá, two congeneric species of trematodes, i.e., *Saccocoelioides*. cf*. lamothei* and *S. orosiensis*, were found in mixed infections in individual poeciliids.

The eleotrid *Gobiomorus* sp. was collected from Lake Nicaragua and three crater lakes. No *Gobiomorus* sp. was infected by parasites, but sample size was very low. *Astyanax* spp. were sampled from the great Nicaraguan lakes (they are absent in the crater lakes) and harbored seven parasite taxa. The sister characiform *Bramocharax* sp. was parasitized by four parasite taxa, all of them shared parasite species with *Astyanax* spp. Individuals of *Bramocharax* sp*.* harbored mixed infections of the monogeneans *Characithecium costaricensis* and an unidentified species of Dactylogyridae. One individual of the haemulid *Pomadasys croco* was sampled in Lake Nicaragua and was infected with the cryptogonomid trematode *Neochasmus ackerti*.

We sequenced the gen 28S for two species of monogeneans: *Characithecium costaricensis* and Dactylogyridae gen. sp., both from characids. Additionally, sequences of the gen cox1 allowed the identification of two species of *Saccocoeiliodes* which caused mixed infections in poecilids. The fragment of the gen 28S of *Gernarchella astyanactis* from the type locality matched with available sequences of the species sampled in characids from Mexico. The sequences of cox2 recovered from larval stages of *Contracaecum* did not allow species identification, as they did not match with available sequences in the GenBank database.

Our literature revision yielded records of 96 parasite taxa in Nicaraguan freshwaters. With the records generated in the present study, there are a total of 101 parasite taxa described in Nicaraguan fish (Supplementary Table S1). Parasite diversity includes species of trematodes, cestodes, monogeneans, acanthocephalans, nematodes, hirudineans, copepods, branchiurans and oribatid mites. Most species correspond to trematodes, with 42 taxa included in 16 families. Sixteen of them are reported as metacercaria. Monogeneans and nematodes are represented by 24 and 20 taxa, respectively. The oribatid mites, hirudineans and branchiurans are considered as rare records since they exhibit low prevalence values in their respective hosts. They are represented by a single taxon and reported only from one or two host species. In addition, three of the 101 parasite taxa are invasive species, namely, the anchor worm, *Lernaea cyprinacea*, the Asian fish tapeworm, *Schizochotyle acheilognathi* and the larval trematode *Centrocestus formosanus*.

The area with the greatest diversity was Lake Nicaragua with 37 parasite taxa, followed by the Black water River (in the Atlantic slope of Nicaragua) with 36 parasite taxa. Sampling was asymmetrical among localities for both fish host species and number of individuals analyzed, resulting in asymmetric patterns of distribution of parasite diversity among waterbodies (fig. 1). Most studies have focused thus far on the lowlands of either the Pacific or the Atlantic slopes, but there is to date no study in the Cordillera Central. Considering the Nicaraguan crater lakes, Lake Xiloá exhibited the greatest diversity, with 20 parasite taxa.

The 101 parasite taxa reported for Nicaragua were found in 51 fish species belonging to 11 families (figs. 2-3, Supplementary Table S2). Therefore, parasite records are still lacking for 55.2% of the freshwater fish species diversity of Nicaragua. Cichlids are the best studied fish group in terms of their parasite fauna, with records from 30 fish species (fig. 2). Three parasite groups are only reported from cichlid hosts: acanthocephalans, hirudineans and branchiurans. Among cichlids, the Midas cichlid harbors the most species-rich fauna of all studied hosts, with 21 parasite taxa. Four species of poeciliids have been studied and 17 parasite taxa were recorded. For characids, including the genera *Astyanax*, *Bramocharax* and *Roeboides*, sampled in the great lakes and streams of the Atlantic slope, the parasite fauna includes 26 parasite taxa. In four species of catfishes (Heptapteridae), 10 parasite taxa have been reported. The remaining fish families exhibited a poor parasite diversity, although we acknowledge that the sampling size for these fish is small: Bryconidae (one taxa), Carcharhinidae (three taxa), Eleotridae (three taxa), Haemulidae (one taxa), Lepisosteidae (two taxa), Megalopidae (one taxa) and Pristidae (two taxa) (Supplementary Table S2).

Discussion

Nicaragua has a natural setting that favors diversification, with large freshwater bodies combined with smaller isolated crater lakes, which collectively are sources of biological endemism. In the last few years, we emphasized our sampling effort in the great Nicaraguan lakes and several crater lakes occurring in Pacific coast of Nicaragua, where cichlids experienced evolutionary success and recent adaptive radiations, similar, albeit to a smaller extent, to the well-known adaptive radiations of cichlids in the East

African Great Lakes (Barluenga *et al.*, 2006; Salzburger *et al.*, 2014; Kautt *et al.*, 2020). The freshwater fish fauna of Nicaragua is constituted by around 104 native species (Froese & Pauly, 2021), of which four siluriforms and nine characiforms are primary fish restricted to freshwater environments, but the rest are secondary freshwater fishes tolerant to different degrees of salinity. The most abundant fish in the Nicaraguan freshwaters are cichlids and poeciliids, which together account for almost 50% of the fish fauna.

The present study represents an additional step to an ongoing effort to characterize the metazoan parasites of Nicaraguan fish (see Santacruz *et al.*, 2020, 2021a,b). Here, we report new data on the parasite fauna of non-cichlid fish hosts. We recorded 17 parasite taxa from seven fish species. The poecilids, widely distributed across all lakes (Astorqui, 1971; Waid *et al.*, 1999), harbored the most parasite species-rich fauna, with six taxa, whereas in characids, we found adult stages typical of their biogeographical core parasite fauna (*sensu* Pérez-Ponce de León & Choudhury, 2005), including the trematode *Genarchella astyanactis* and the monogenean *Characithecium costaricensis*. Interestingly, the eleotrid *Gobiomorus* sp. (introduced in crater Lake Apoyo, Waid *et al.*, 1999), harbored a poor parasite fauna, in accordance with an invasive species, which can loss parasites after a new colonization (Roche *et al*., 2010).

In Nicaragua, considering all existing parasite records, the Midas cichlid species complex *Amphilophus* spp. possess the highest parasite species richness, with 26 taxa. The large parasite species diversity of these cichlids agrees with the observed diversity in Middle American cichlids of the tribe Heroini (Razo-Mendivil *et al.*, 2009, 2010, 2015). This high parasite species richness in cichlids may respond to their remarkable dominance in Middle American freshwaters, and the great number of endemisms in the lacustrine systems. However, the differences may also account for a sampling bias given the large interest these fishes raise. Overall, Middle American cichlids are parasitized by a large diversity of taxa, particularly helminth parasites (see Vidal-Martínez *et al.*, 2001a).

The parasite fauna of Nicaraguan freshwater fishes is mainly of Neotropical origin, only few records have been made on Nearctic fish hosts, such as gars, which extend their southernmost distribution range to the San Juan river, which joins Lake Nicaragua with the Atlantic slope. Trematodes represent the dominant component of the parasite fauna; the list including taxa from 16 families, reported in 39 fish species, both as adults or as metacercariae. Most of the trematode fauna of Nicaragua is shared with that of Mexican freshwaters, where Neotropical fishes reach their northernmost distributional range (see Pérez-Ponce de León & Choudhury, 2005).

The freshwater fish parasite fauna of Lower Central America

The data gathered on the metazoan parasites of freshwater fishes of Nicaragua reveal a species-rich fauna, with a total of 101 parasite taxa belonging to eight parasitic groups, and these parasites infect 51 host species (less than 50% of the fish fauna). This makes Nicaragua the most thoroughly studied region of Central America. In contrast, other areas in Lower Central America with higher fish richness, e.g. Costa Rica and Panama, parasite surveys reported lower parasite species richness (see Supplementary Table S3 and S4). To the best of our knowledge, in Costa Rica at least 44 of the 178 freshwater fish species (Froese & Pauly, 2021) have been studied for parasites to a certain extent, and 59 parasite taxa have been reported (Chandler *et al.*, 1995; Choudhury *et al.*, 2002, 2006; López-Caballero *et al.*, 2009; Sandlund *et al.*, 2010; Arguedas Cortés *et al.*, 2010; Pinacho-Pinacho *et al.*, 2015, 2020, 2021; Tkach & Curran, 2015; Andrade-Gómez *et al.*, 2017, 2019; De Chambrier *et al.*, 2017; López-Jiménez *et al.*, 2018; Curran *et al.*, 2018; Sereno-Uribe *et al.*, 2019; Briosio-Aguilar *et al.*, 2019; González-García *et al.*, 2021) (Supplementary Table S3). In Panama, 25 of the 213 freshwater fish species (Froese & Pauly, 2021) have been studied for parasites, and 63 parasite taxa have been reported (Sogandares-Bernal, 1955; Mendoza-Franco *et al.*, 2007, 2008, 2009a; b; Mendoza-Franco & Reina, 2008; Kritsky *et al.*, 2009; Roche *et al.*, 2010; Choudhury *et al.*, 2013, 2017) (Supplementary Table S4). Nicaragua, along with Costa Rica and Panama comprise a geographical region known as Lower Central America (LCA). Overall, the freshwater fish fauna of LCA is comprised by at least 176 parasite taxa, although the proportion of fish species analyzed thus far is still relatively low. This clearly indicates that the inventory of the freshwater fish parasite fauna is far from complete. However, some interesting biogeographical patterns emerged, particularly when adult stages are considered, since larval forms might obscure patterns as they can be dispersed with their definitive hosts, usually fish-eating birds. The parasite fauna of LCA is mainly composed by adult parasites, with 127 taxa out of the 173 reported. The remaining taxa are larval forms that complete their life cycle in another vertebrate. Most of the larval forms are found in fish-eating birds (32 of the 42 taxa), while the other use either larger fish, mammals, or reptiles as definitive hosts. Current data shows that only five adult taxa, i.e., the trematodes *Acanthostomum minimum, Oligogonotylus manteri*, *Prosthenhystera obesa, Saccocoelioides cichlidorum*, and the nematode *Rhabdochona* sp., are shared across all LCA. However, this might be a sampling artifact, and the result of incomplete sampling of hosts or inaccurate species identifications. Interestingly, at least 29 parasite taxa are found only in freshwater fishes in this region, accounting for 16.7% of endemism.

In summary, the results of our study highlight the diversity of parasites in freshwater systems of Nicaragua, and show the spatial distribution of the sampling effort, mainly in the lowlands of the west and east of the Cordillera Central. Our data add information to the inventory of the parasite fauna of freshwater fishes in this important region. The biogeographical event referred as the Great American Biotic Interchange (GABI) require the use of different taxa to better explain the major patterns of distribution derived from the formation of the land bridge. We acknowledge that the inventory of the freshwater fish parasite fauna of Nicaragua is still far for complete, but the current information is very useful for better understanding the role of GABI in the diversification of both hosts and parasites. In addition, we totally concur with Poulin *et al.* (2020) in that the research programs on fish parasite discovery require an improvement of its efficiency to achieve a better understanding of parasite diversity patterns, which is central to comprehend the evolutionary-based classification of living forms on the planet. In our study, we targeted a geographical hotspot of fish biodiversity, and we used molecular tools to study parasite genetic diversity in cases where morphology was not enough to establish proper species identifications; for the descriptions of new taxa, we followed an integrative taxonomy approach that considered traits such as morphology, DNA, host association and geographical distribution; this sets a standard quality criterion for new species descriptions. Poulin *et al.* (2020) clearly stated that since taxonomy and systematics underpin all other research in ecology and evolution, and applied science related, for example, with fisheries and aquaculture, it is necessary first to discover parasite species and then to characterize host-parasite associations. On top of that, our study on the parasite fauna of Nicaraguan freshwater fishes is part of a larger project aimed at understanding the potential role of parasites in the diversification of the Midas cichlid species complex which mirrors the case of adaptive radiations of cichlids in African lakes. Information on the parasite fauna of these fish shed light on the factors that determine cases of sympatric speciation.

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Conflict of interest declaration. None

Ethical standards. The authors assert that host were studied following all applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Fish were euthanized and killed following procedures of the American Veterinary Medical Association. Hosts were collected under the collecting permit No. 001-012015 issued by the Nicaraguan Ministry of Natural Resources MARENA.

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Figure legends

Figure 1. Map of Nicaragua. The sites for the records of host-parasites reported from freshwater systems in Nicaragua. In parenthesis next to the site name, the number of parasite taxa reported for each site is indicated. Cl. Crater lake, L. Lake, C. Creek, R. River.

Figure 2. **Parasites in freshwater fishes of Nicaragua**. Total number of parasite taxa (above) and the number of fish host species and host family (below) where had been reported.

Figure 3. Photomicrographs of representative parasites in freshwater fishes from the Nicaraguan lakes. The trematodes: (a) Criptogonomidae gen. sp., (b) *Oligogonotylus manteri*, (c) *Saccocoeilioides* sp., (d) *Genarchella astynactys*, (e) *Crassicutis cichlasomae*, (f) Trematoda, (g) Strigeidae gen. sp., (h) *Ascocotyle* sp., (i) *Posthodiplostomum* sp. 2, (j) *Posthodiplostomum* sp. 1, (k) Heterophyidae gen. sp. (l) The monogenan *Sciadicleithrum mexicanum*, (m) the cestode *Cichlidocestus janikae*, (n) the nematode *Goezia* sp. and (o) the hirudinean *Myzobdella* .

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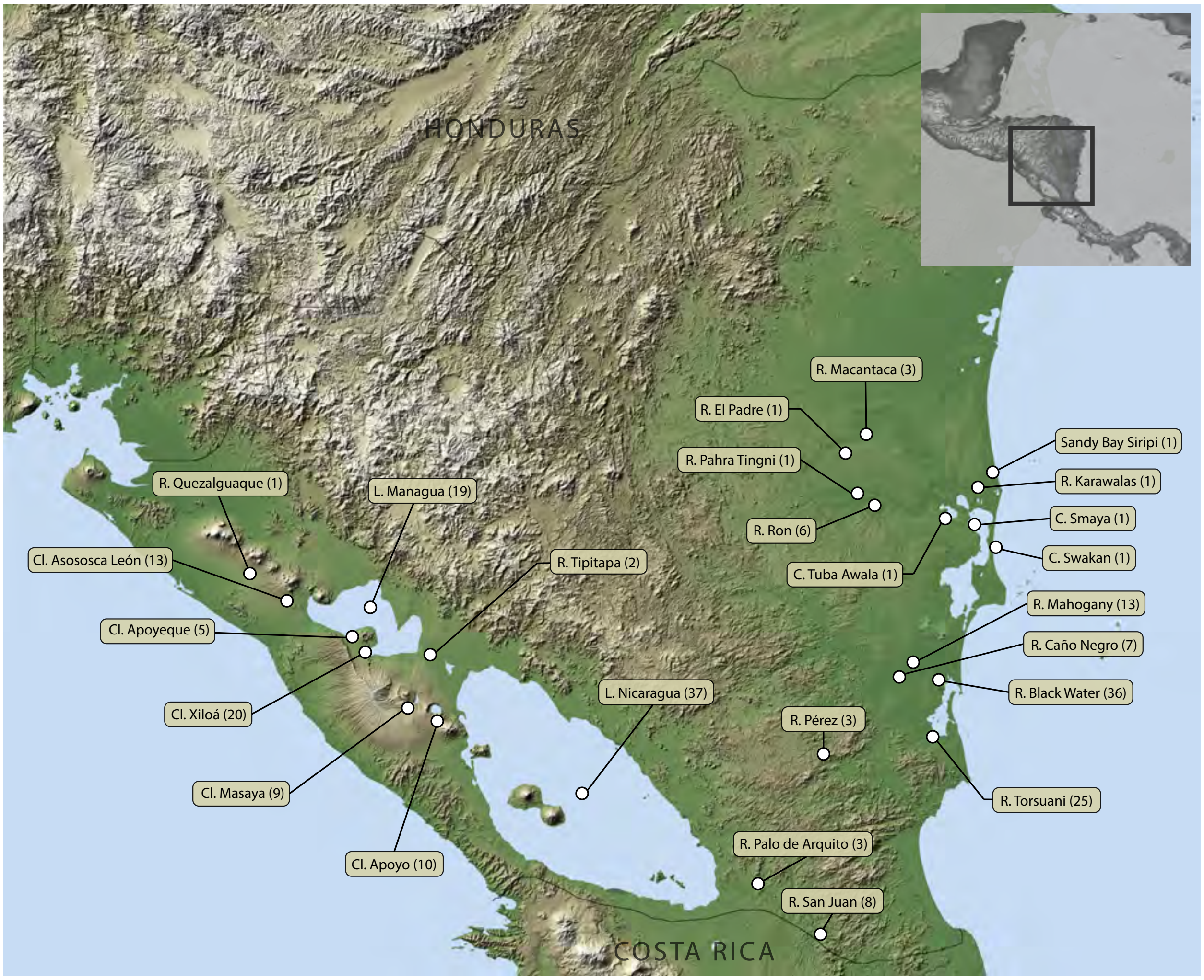

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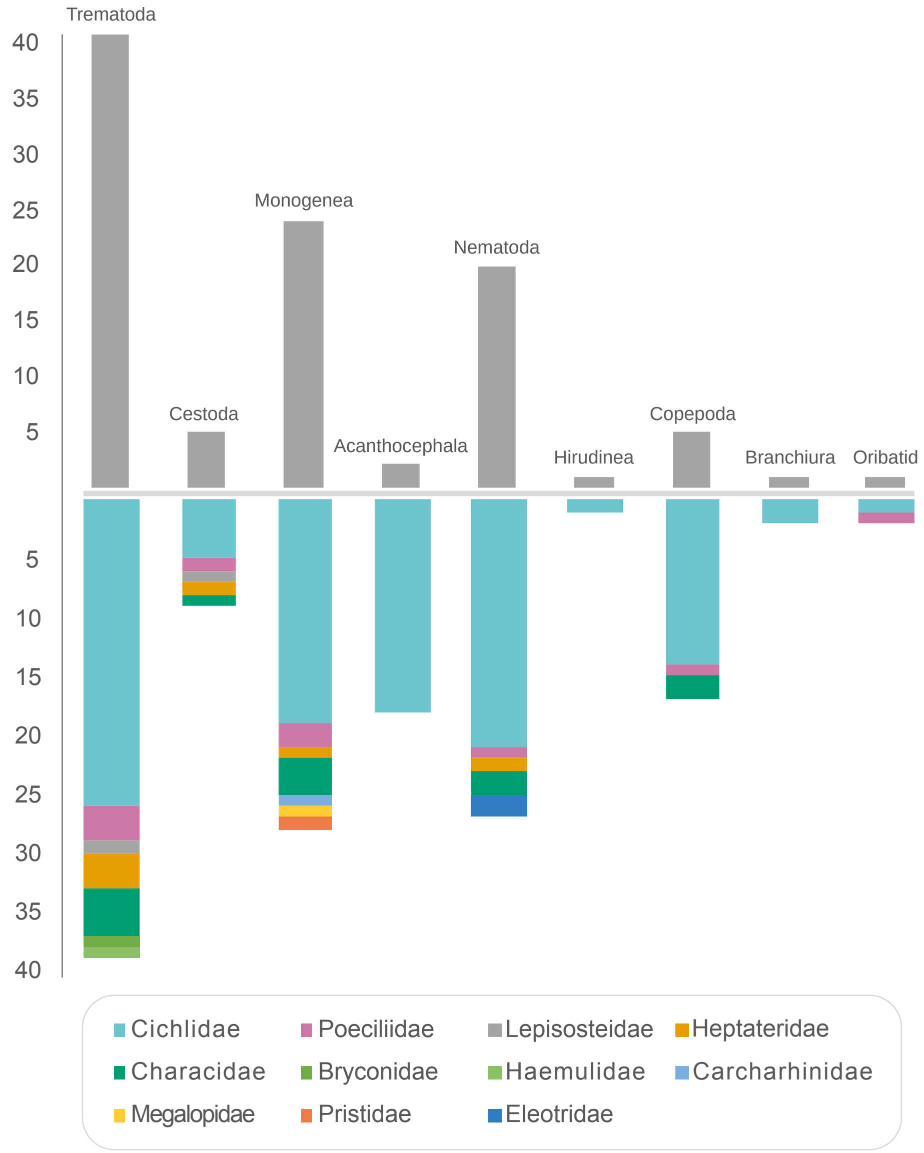

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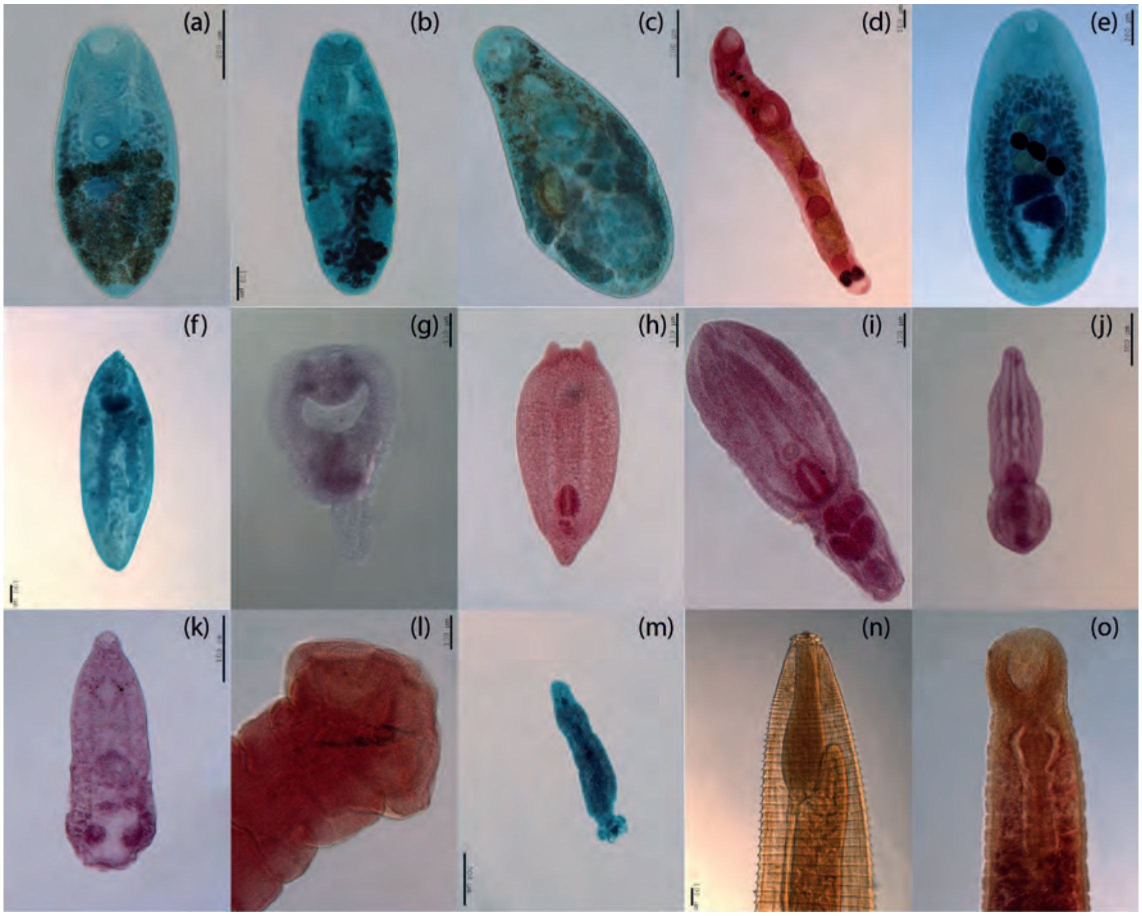
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Table 1. Fish species analyzed for metazoan parasites in the two great lakes and four crater lakes of Nicaragua, and parasite records within lakes presented in alphabetical order per host species, with some ecological parameter data of the infection. S, stage, adult A or larvae L. Si, site of infection.

Lake and host	N hosts	Parasite taxa	Si	S	Prevalence %	Mean intensity ± SD
Nicaragua						
Astyanax sp.	31	*Centrocestus formosanus*	gills	L	3.2	1
		Characithecium costaricensis	gills	A	50	18.4 ± 14.8
		Contracaecum sp. 1	body cavity	L	23.3	1.8 ± 1.4
		Lernaea cyprinacea	skin	A	3.2	1
Bramocharax sp.	7	*Characithecium costaricensis*	gills	A	1.5	5.5 ± 4.9
		Dactylogyridae gen. sp.	gills	A	14.3	1
		Ergasilidae gen. sp.	gills	A	60	1
		Lernaea cyprinacea	skin	A	14.2	1
Gobiomorus sp.	1	—	—	A	—	—
Poecilia sp.	7	Cyclophyllidea gen. sp.	liver & body cavity	L	28.5	1
		Ergasilidae gen. sp.	gills	A	16.6	1
		Oribatide gen. sp.	body cavity	A	14.2	5
		Posthodiplostomum sp. 1	muscle	L	71.4	5.8 ± 7.6
		Saccocoelioides orosiensis	gut	A	71.4	5.4 ± 6.9
Roeboides sp.	1	—	—		—	—
Pomadasys croco	1	*Neocasmus ackerti*	gut	A	100	33
Rhamdia sp.	2	*Clinostomum* sp.	body cavity	L	50	1
		Proteocephalidae gen. sp.	gut	A	50	1
Managua						
Astyanax sp.	53	*Austrodiplostomum compactum*	eye	L	13.2	1.5 ± 1.5
		Centrocestus formosanus	gills	L	5.4	1

		Characithecium costaricensis	gills	A	55.6	27.6 ± 17.3
		Contracaecum sp. 1	body cavity	L	56.9	4.1 ± 4.5
		Ergasilidae gen. sp.	gills	A	11.1	1
		Genarchella astyanactis	gut	A	5.7	2 ± 1
		Lernaea cyprinacea	skin	A	11.3	1
Bramocharax sp.	1	*Characithecium costaricensis*	gills	A	100.0	31
Poecilia sp.	6	*Posthodiplostomum* sp. 1	muscle	L	100.0	62.6 ± 48.1
		Saccocoleioides spp.	gut	A	50.0	0.8 ± 1.1
Asososca León						
Poecilia sp.	4	*Acusicola margulisae*	gills	A	100	26 ± 22.6
		Saccocoelioides cf. *lamothei*	gut	A	100	13 ± 4.5
		Saccocoelioides orosiensis	gut	A		
		Saccocoleioides spp.	gut	A		
Xiloá						
Gobiomorus sp.	4	—	—	—	—	—
Poecilia sp.	12	Ergasilidae gen. sp.	gills	A	100	1
		Saccocoelioides cf. *lamothei*	gut	A	58.3	2.1 ± 1.7
		Saccocoelioides orosiensis	gut	A		
		Saccocoleioides spp.	gut	A		
Masaya						
Gobiomorus sp.	1	*Contracaecum* sp. 1	body cavity	L	100	5
Apoyo						
Gobiomorus sp.	3	—	—	—	—	—
Poecilia sp.	2	—	—	—	—	—

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Filling the knowledge gap of Middle American freshwater fish parasite biodiversity: Metazoan parasite fauna of Nicaragua

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Supplementary Table S1. List for freshwater fish parasites of Nicaragua. Some parasite names are in accordance with the current acceptance in the literature, not as they were presented in the original reference. S, stage, adult A or larvae L. The asterisk indicates an invasive species. In bold the species reported in this study.

Parasite Family	Parasite taxa	S	Host species	Locality	Reference
Trematoda					
Acanthostomatidae Poche, 1926	*Acanthostomum astorquii*	A	*Rhamdia nicaraguensis*	Lake Nicaragua	Watson, 1976
	Acanthostomum gnerii	A	*Rhamdia managuensis*	Lake Nicaragua	Watson, 1976
			Rhamdia nicaraguensis	Lake Nicaragua	Watson, 1976
	Acanthostomum minimum (=Stunkardiella minima)	A	*Vieja maculicauda*	Caño Marañon	Aguirre-Macedo *et al*., 2001a
Allocreadiidae Losos, 1902	*Auriculostoma astyanace*	A	*Astyanax fasciatus*	Torsuani River	Aguirre-Macedo *et al*., 2001b
			Astyanax fasciatus	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001b
			Astyanax fasciatus	Loonku Creek, Bluefields	Scholz et al., 2004
			Astyanax fasciatus	Torsuani River	Scholz et al., 2004
Alloglossidiidae Hernández-Mena, Mendoza-Garfías, Ornelas-García & Pérez-Ponce de León, 2016	*Magnivitellum simplex*	A	*Astyanax fasciatus*	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001b
			Astyanax fasciatus	Torsuani River	Aguirre-Macedo *et al*., 2001b
Callodistomidae Poche, 1926	*Prosthenhystera obesa*	A	*Astyanax fasciatus*	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001b
	Prosthenhystera caballeroi	A	*Astyanax aeneus*	Quezalguaque River near Telica	Tkach et al., 2015

Clinostomidae Lühe, 1901	***Ithyoclinostomum yamaguti*** *(= Clinostomum* sp.*)*	L	*Parachromis managuensis*	Mahogany River	Aguirre-Macedo et al., 2001a
			Parachromis managuensis	Caño Negro	Aguirre-Macedo et al., 2001a
			Hypsophrys nematopus	Lake Nicaragua	Santacruz *et al*., 2021
			Hypsophrys nicaraguensis	Lake Nicaragua	Santacruz *et al*., 2021
			Parachromis managuensis	Mahogany River	Aguirre-Macedo et al., 2001a
			Parachromis managuensis	Caño Negro	Aguirre-Macedo et al., 2001a
			Parachromis sp.	Lake Nicaragua	Santacruz *et al*., 2021
	Clinostomum sp.	L	*Rhamdia* sp.	Lake Nicaragua	**This study**
Crytogonimidae Ward, 1917	Cryptogonimidae gen. sp.	A	*Parachromis* sp.	Lake Managua	Santacruz *et al*., 2021
			Poecilia velifera	Caño Marañon	Aguirre-Macedo et al., 2001a
	Neochasmus ackerti	A	*Pomadasys croco*	Lake Nicaragua	Watson, 1976; **this study**
	Oligogonotylus manteri	A	*Amphilophus amarillo*	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus astorquii	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus chancho	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus citrinellus	Lake Managua	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Asosca León	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Apoyeque	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Masaya	Santacruz *et al*., 2021
			Amphilophus citrinellus	Lake Nicaragua	Watson, 1976; Santacruz *et al*., 2021
			Amphilophus globosus	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Managua	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Nicaragua	Watson, 1976; Santacruz *et al*., 2021
			Amphilophus sagittae	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus xiloaensis	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus zaliosus	Crater lake Apoyo	Santacruz *et al*., 2021
			Cribroheros longimanus	Crater lake Xiloá	Santacruz *et al*., 2021
			Cribroheros rostratus	Lake Managua	Santacruz *et al*., 2021
			Cribroheros rostratus	Crater lake Xiloá	Santacruz *et al*., 2021
			Cribroheros rostratus	Lake Nicaragua	Watson, 1976; Santacruz *et al*., 2021
			Hypsophrys nematopus	Crater lake Xiloá	Santacruz *et al*., 2021
			Hypsophrys nematopus	Crater lake Masaya	Santacruz *et al*., 2021
			Hypsophrys nematopus	Lake Nicaragua	Santacruz *et al*., 2021
			Hypsophrys nematopus	Lake Managua	Santacruz *et al*., 2021
			Hypsophrys nicaraguensis	Crater lake Xiloá	Santacruz *et al*., 2021

			Hypsophrys nicaraguensis	Lake Nicaragua	Watson, 1976; Santacruz *et al*., 2021
			Parachromis managuensis	Lake Nicaragua	Watson, 1976; Santacruz *et al*., 2021
			Parachromis managuensis	Puente Chino	Aguirre-Macedo *et al*., 2001b
			Parachromis sp.	Lake Managua	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Asosca León	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Apoyeque	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Xiloá	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Apoyo	Santacruz *et al*., 2021
			Vieja maculicauda	Lake Nicaragua	Watson, 1976;
			Vieja maculicauda	Caño Marañon	Aguirre-Macedo *et al*., 2001a; Aguirre-Macedo *et al*., 2001b
			Vieja maculicauda	Torsuani River	Aguirre-Macedo *et al*., 2001a; Aguirre-Macedo *et al*., 2001b
Derogenidae Nicoll, 1910	***Genarchella astyanactys (=Paravitellotrema astyanactis)***	A	*Astyanax fasciatus*	Lake Nicaragua at Tepetate^	Watson, 1976
			Astyanax sp.	Lake Managua	**This study**
	Genarchella thorsoni (=Paravitellotrema thorsoni)	A	*Rhamdia managuensis*	Lake Nicaragua	Watson, 1976
Diplostomatidae Poirier, 1886	***Austrodiplostomum compactum (=Diplostomum compactum)***	L	*Amphilophus citrinellus*	Lake Managua	Santacruz *et al*., 2021
			Astyanax sp.	Lake Managua	**This study**
			Cribroheros alfari	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a
			Cribroheros rostratus	Lake Managua	Santacruz *et al*., 2021
			Parachromis sp.	Lake Managua	Santacruz *et al*., 2021
	Posthodiplostomum minimum	L	*Amatitlania nigrofasciata*	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a
			Herotilapia multispinosa	Puente Chino	Aguirre-Macedo *et al*., 2001a
			Parachromis managuensis	Caño Negro	Aguirre-Macedo *et al*., 2001a
			Parachromis managuensis	Mahogany River	Aguirre-Macedo *et al*., 2001a
			Poecilia velifera	Caño Marañon	Aguirre-Macedo *et al*., 2001a
			Vieja maculicauda	Torsuani River	Aguirre-Macedo *et al*., 2001a
	***Posthodiplostomum* sp. 1**	L	*Hypsophrys nematopus*	Lake Nicaragua	Santacruz *et al*., 2021
			Hypsophrys nematopus	Crater lake Masaya	Santacruz *et al*., 2021
			Poecilia sp.	Lake Nicaragua	**This study**
	Posthodiplostomum sp. 2	L	*Amatitlania nigrofasciata*	Lake Nicaragua	Santacruz *et al*., 2021
	Uvulifer sp.	L	*Amatitlania nigrofasciata*	Puente Chino	Aguirre-Macedo *et al*., 2001a
			Amatitlania siquia	Río Perez	López-Jiménez *et al*., 2017

			Archocentrus centrarchus	Palo de Arquito	López-Jiménez *et al*., 2017
			Astyanax fasciatus	Torsuani River	Aguirre-Macedo *et al*., 2001a
			Cribroheros alfari	Puente Chino	Aguirre-Macedo *et al*., 2001a
			Cribroheros longimanus	San Carlos	López-Jiménez *et al*., 2017
			Herotilapia multispinosa	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a
			Herotilapia multispinosa	Mahogany River	Aguirre-Macedo *et al*., 2001a
			Hypsophrys sp.	Palo de Arquito	López-Jiménez *et al*., 2017
			Vieja maculicauda	Caño Marañon	Aguirre-Macedo *et al*., 2001a
			Vieja maculicauda	Caño Negro	Aguirre-Macedo *et al*., 2001a
			Vieja maculicauda	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a
			Vieja maculicauda	Mahogany River	Aguirre-Macedo *et al*., 2001a
			Vieja maculicauda	Torsuani River	Aguirre-Macedo *et al*., 2001a
	Uvulifer spinatus	L	*Alfaro cultratus*	Blue Fields	López-Jiménez *et al*., 2017
			Poecilia mexicana	Río Perez	López-Jiménez *et al*., 2017
Gorgoderidae Looss, 1899	*Phyllodistomum romualdae*	A	*Archocentrus centrarchus*	Finca Espavel, San Carlos	Pinacho-Pinacho *et al*., 2021
	Phyllodistomum scotti	A	*Rhamdia nicaraguensis*	Finca Espavel, San Carlos	Pinacho-Pinacho *et al*., 2021
			Rhamdia nicaraguensis	Palo de Arquito	Pinacho-Pinacho *et al*., 2021
Haploporidae Nicoll, 1914	*Saccocoelioides cichlidorum (=Culuwiya cichlidorum**)*	A	*Vieja maculicauda*	Torsuani River	Andrade-Gómez *et al*., 2019
			Vieja maculicauda	Caño Marañon	Aguirre-Macedo & Scholz, 2005; Andrade-Gómez et al., 2017
			Vieja maculicauda	Torsuani River	Aguirre-Macedo & Scholz, 2005; Andrade-Gómez et al., 2017
	Saccocoelioides* cf. *lamothei	A	*Amphilophus citrinellus*	Crater lake Asosca León	Santacruz *et al*., 2021
			Hypsophrys nematopus	Lake Nicaragua	Santacruz *et al*., 2021
			Oreochromis sp.	Crater lake Asosca León	Santacruz *et al*., 2021
			Poecilia sp.	Crater lake Asosca León	**This study**
			Poecilia sp.	Crater lake Xiloá	**This study**
	Saccocoelioides orosiensis	A	*Amatitlania nigrofasciata*	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus astorquii	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus chancho	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus sagittae	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus xiloaensis	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus zaliosus	Crater lake Apoyo	Santacruz *et al*., 2021
			Hypsophrys nematopus	Crater lake Xiloá	Santacruz *et al*., 2021
			Hypsophrys nicaraguensis	Crater lake Xiloá	Santacruz *et al*., 2021

			Oreochromis sp.	Crater lake Asosca León	Santacruz *et al*., 2021
			Poecilia sp.	Lake Nicaragua	**This study**
			Poecilia sp.	Crater lake Asosca León	**This study**
			Poecilia sp.	Crater lake Xiloá	**This study**
	Saccocoelioides sogandaresi	A	*Poecilia velifera*	Caño Marañon	Aguirre-Macedo *et al*., 2001b
	Saccocoelioides sp.	A	*Brycon* sp.	Torsuani River	Andrade-Gómez *et al*., 2017
			Amphilophus astorquii	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus chancho	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus citrinellus	Lake Managua	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Asosca León	Santacruz *et al*., 2021
			Amphilophus flaveolus	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus globosus	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus sagittae	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus zaliosus	Crater lake Apoyo	Santacruz *et al*., 2021
			Hypsophrys nematopus	Lake Nicaragua	Santacruz *et al*., 2021
			Oreochromis sp.	Lake Managua	Santacruz *et al*., 2021
			Oreochromis sp.	Crater lake Asosca León	Santacruz *et al*., 2021
			Parachromis sp.	Lake Managua	Santacruz *et al*., 2021
			Poecilia sp.	Lake Managua	**This study**
			Poecilia sp.	Crater lake Asosca León	**This study**
			Poecilia sp.	Crater lake Xiloá	**This study**
			Roeboides guatemalensis	Lake Nicaragua	Watson, 1976
	Saccocoelioides sp. 1	A	*Vieja maculicauda*	Torsuani River	Aguirre-Macedo *et al*., 2001b
			Vieja maculicauda	Caño Marañon	Aguirre-Macedo *et al*., 2001b
	Saccocoelioides sp. 2	A	*Astyanax fasciatus*	Torsuani River	Aguirre-Macedo *et al*., 2001b
	Saccocoelioides tkachi	A	*Astyanax aeneus*	Palo de Arquito	Andrade-Gómez *et al*., 2019
			Astyanax aeneus	Río Perez	Andrade-Gómez *et al*., 2019
			Astyanax aeneus	Torsuani River	Andrade-Gómez *et al*., 2019
Heterophyidae Leiper, 1909	*Ascocotyle diminuta*	L	*Poecilia velifera*	Caño Marañon	Aguirre-Macedo *et al*., 2001a
	Ascocotyle mollienisicola	L	*Poecilia velifera*	Caño Marañon	Aguirre-Macedo *et al*., 2001a
	Ascocotyle nana	L	*Vieja maculicauda*	Torsuani River	Aguirre-Macedo *et al*., 2001a
	Ascocotyle pindoramensis	L	*Amphilophus labiatus*	Lake Nicaragua	Santacruz *et al*., 2021
			Parachromis sp.	Lake Managua	Santacruz *et al*., 2021
	Ascocotyle tenuicollis	L	*Astyanax fasciatus*	Torsuani River	Aguirre-Macedo *et al*., 2001a
			Poecilia velifera	Torsuani River	Aguirre-Macedo *et al*., 2001a
			Vieja maculicauda	Torsuani River	Aguirre-Macedo *et al*., 2001a
	Centrocestus formosanus*	L	*Astyanax* sp.	Lake Nicaragua	**This study**
			Astyanax sp.	Lake Managua	**This study**
	Heterophyidae gen. sp.	L	*Cribroheros alfari*	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a

			Herotilapia multispinosa	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a
			Oreochromis sp.	Lake Managua	Santacruz *et al*., 2021
			Parachromis managuensis	Mahogany River	Aguirre-Macedo *et al*., 2001a
Lepocreadiidae Odhner, 1905	*Crassicutis cichlasomae*	A	*Archocentrus centrarchus*	Lake Nicaragua	Santacruz *et al*., 2021
			Amatitlania nigrofasciata	Lake Nicaragua	Santacruz *et al*., 2021
			Amatitlania nigrofasciata	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus amarillo	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus astorquii	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus chancho	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus citrinellus	Lake Managua	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Asosca León	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Apoyeque	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Masaya	Santacruz *et al*., 2021
			Amphilophus citrinellus	Lake Nicaragua	Watson, 1976; Santacruz *et al*., 2021
			Amphilophus flaveolus	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus globosus	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Managua	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Nicaragua	Watson, 1976; Santacruz *et al*., 2021
			Amphilophus sagittae	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus xiloaensis	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus zaliosus	Crater lake Apoyo	Santacruz *et al*., 2021
			Cribroheros longimanus	Crater lake Xiloá	Santacruz *et al*., 2021
			Cribroheros longimanus	Crater lake Masaya	Santacruz *et al*., 2021
			Cribroheros rostratus	Lake Managua	Santacruz *et al*., 2021
			Cribroheros rostratus	Crater lake Xiloá	Santacruz *et al*., 2021
			Cribroheros rostratus	Lake Nicaragua	Watson, 1976; Santacruz *et al*., 2021
			Cryptoheros spilurus	Lake Nicaragua	Watson, 1976
			Hypsophrys nicaraguensis	Crater lake Xiloá	Santacruz *et al*., 2021
			Hypsophrys nematopus	Lake Nicaragua	Santacruz *et al*., 2021
			Hypsophrys nicaraguensis	Lake Nicaragua	Watson, 1976; Santacruz *et al*., 2021
			Parachromis managuensis	Lake Nicaragua	Watson, 1976; Santacruz *et al*., 2021
			Parachromis sp.	Lake Managua	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Apoyeque	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Apoyo	Santacruz *et al*., 2021

			Vieja maculicauda	Caño Marañon	Aguirre-Macedo et al., 2001b; Santacruz et al., 2021
Macroderoididae McMullen, 1937	*Perezitrema* sp. *(=Allomacroderoides lepisostei)*	A	*Atractosteus tropicus*	Lake Nicaragua	Watson, 1976
Opisthorchiidae Losss, 1899	*Cladocystis trifolium*	A	*Parachromis managuensis*	Mahogany River	Aguirre-Macedo *et al.*, 2001a
Proterodiplostomidae Dubois, 1936	Proterodiplostomidae gen sp.	A	*Astyanax fasciatus*	Torsuani River	Aguirre-Macedo *et al.*, 2001a
			Herotilapia multispinosa	Loonku Creek, Bluefields	Aguirre-Macedo *et al.*, 2001a
			Rhamdia managuensis	Puente Chino	Aguirre-Macedo *et al.*, 2001a
Strigeidae Railliet, 1919	Strigeidae gen sp.	L	*Hypsophrys nematopus*	Lake Nicaragua	Santacruz *et al.*, 2021
Cestoda					
Cyclophyllidea van Beneden in Braun, 1900	**Cyclophylidea gen. sp.**	L	*Poecilia* sp.	Lake Nicaragua	**This study**
Proteocephalidae La Rue, 1911	*Cichlidocestus janikae*	A	*Amphilophus citrinellus*	Lake Nicaragua	Santacruz *et al.*, 2021
			Hypsophrys nicaraguensis	Lake Nicaragua	Santacruz *et al.*, 2021
	Proteocephalidae gen. sp.	A	*Astyanax fasciatus*	Torsuani River	Aguirre-Macedo *et al.*, 2001a
			Herotilapia multispinosa	Mahogany River	Aguirre-Macedo *et al.*, 2001a
			Parachromis managuensis	Mahogany River	Aguirre-Macedo *et al.*, 2001a
			Rhamdia sp.	Lake Nicaragua	**This study**
	Proteocephalus gaspari	A	*Atractosteus tropicus*	Lake Managua	Chambrier & Vaucher, 1984
Bothriocephalidae Blanchard, 1949	*Schyzocotyle acheilognathi*	A	*Amphilophus citrinellus*	Lake Managua	Santacruz *et al.*, 2021
			Parachromis sp.	Crater lake Xiloá	Santacruz *et al.*, 2021
Monogenea					
Dactylogyridae Bychowsky, 1933	*Aphanoblastella travassosi*	A	*Rhamdia nicaraguensis*	Macantaca River	Mendoza-Franco *et al.*, 2003
			Rhamdia nicaraguensis	Black Water River	Mendoza-Franco *et al.*, 2003
			Rhamdia nicaraguensis	Smaya Creek	Mendoza-Franco *et al.*, 2003
			Rhamdia nicaraguensis	Loonku Creek, Bluefields	Mendoza-Franco *et al.*, 2003
			Rhamdia nicaraguensis	Puente Chino	Mendoza-Franco *et al.*, 2003
	Ancyrocephalinae sp.	A	*Astyanax fasciatus*	Ron River	Mendoza-Franco *et al.*, 2003
	Characithecium costaricensis *(= Urocleroides costaricensis)*	A	*Astyanax* sp.	Lake Managua	**This study**
			Astyanax sp.	Lake Nicaragua	**This study**
			Astyanax fasciatus	Black Water River	Mendoza-Franco *et al.*, 2003
			Astyanax fasciatus	Torsuani River	Mendoza-Franco *et al.*, 2003
			Bramocharax sp.	Lake Managua	**This study**
			Bramocharax sp.	Lake Nicaragua	**This study**
	*Cichlidogyrus sclerosus**	A	*Oreochromis* sp.	Crater lake Asosca León	Santacruz *et al.*, 2021
	Cleidodiscus chavarriai *(=Ameloblastella chavarriai)*	A	*Rhamdia nicaraguensis*	Macantaca River	Mendoza-Franco *et al.*, 2003
			Rhamdia nicaraguensis	Puente Chino	Mendoza-Franco *et al.*, 2003

Cleidodiscus strombicirrus (=Urocleroides strombicirrus)	A	*Astyanax fasciatus*	Ron River	Mendoza-Franco *et al*., 2003
		Astyanax fasciatus	Walpatara bridge	Mendoza-Franco *et al*., 2003
Dactylogyridae gen. sp.	A	*Bramocharax* sp.	Lake Nicaragua	**This study**
Dermophthirius maccallumi	A	*Carcharhinus leucas*	Río San Juan, San Juan	Watson & Thorson, 1976
Gussevia herotilapiae	A	*Herotilapia multispinosa*	Mahogany river	Vidal-Martínez et al., 2001
Heteronchocotyle leucas	A	*Carcharhinus leucas*	Río San Juan, San Juan	Watson & Thorson, 1976
		Carcharhinus leucas	Zapatera Island, Lake Nicaragua	Watson & Thorson, 1976
Sciadicleithrum bicuense	A	*Amatitlania nigrofasciata*	Loonku Creek, Bluefields	Vidal-Martínez et al., 2001
Sciadicleithrum bravohollisae	A	*Vieja maculicauda*	Karawalas river	Mendoza-Franco *et al*., 2003
		Vieja maculicauda	Torsuani River	Mendoza-Franco *et al*., 2003
		Vieja maculicauda	Pahra Tingni River	Mendoza-Franco *et al*., 2003
		Vieja maculicauda	Sandy Bay Sirpi community	Mendoza-Franco *et al*., 2003
		Vieja maculicauda	Tuba Awala Creek	Mendoza-Franco *et al*., 2003
		Vieja maculicauda	Torsuani River	Mendoza-Palmero et al., 2017
Sciadicleithrum maculicaudae	A	*Vieja maculicauda*	Caño Marañon	Vidal-Martínez *et al*., 2001
Sciadicleithrum meekii	A	*Amatitlania nigrofasciata*	Loonku Creek, Bluefields	Vidal-Martínez *et al*., 2001
		Amatitlania nigrofasciata	Ron River	Mendoza-Franco *et al*., 2003
Sciadicleithrum mexicanum	A	*Amphilophus amarillo*	Crater lake Xiloá	Santacruz *et al*., 2021
		Amphilophus astorquii	Crater lake Apoyo	Santacruz *et al*., 2021
		Amphilophus chancho	Crater lake Apoyo	Santacruz *et al*., 2021
		Amphilophus citrinellus	Lake Nicaragua	Santacruz *et al*., 2021
		Amphilophus citrinellus	Lake Managua	Santacruz *et al*., 2021
		Amphilophus citrinellus	Crater lake Masaya	Santacruz *et al*., 2021
		Amphilophus flaveolus	Crater lake Apoyo	Santacruz *et al*., 2021
		Amphilophus sagittae	Crater lake Xiloá	Santacruz *et al*., 2021
		Amphilophus xiloaensis	Crater lake Xiloá	Santacruz *et al*., 2021
		Amphilophus zaliosus	Crater lake Apoyo	Santacruz *et al*., 2021
		Mayaheros urophthalmus	Swakan Creek	Mendoza-Franco *et al*., 2003
		Parachromis dovii	Ron River	Mendoza-Franco *et al*., 2003
		Parachromis dovii	Torsuani River	Mendoza-Franco *et al*., 2003
		Parachromis managuensis	Caño Negro	Vidal-Martínez *et al*., 2001
		Parachromis managuensis	Puente Chino	Vidal-Martínez *et al*., 2001
		Parachromis managuensis	Mahogany river	Vidal-Martínez *et al*., 2001
		Parachromis managuensis	Macantaca River	Mendoza-Franco *et al*., 2003
		Parachromis sp.	Lake Nicaragua	Santacruz *et al*., 2021
		Parachromis sp.	Lake Managua	Santacruz *et al*., 2021
		Parachromis sp.	Crater lake Asosca León	Santacruz *et al*., 2021

			Parachromis sp.	Crater lake Apoyeque	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Apoyo	Santacruz *et al*., 2021
			Tomocichla tuba	Torsuani River	Mendoza-Franco *et al*., 2003
			Vieja maculicauda	Torsuani River	Vidal-Martínez *et al*., 2001; Mendoza-Franco *et al*., 2003
			Vieja maculicauda	Caño Marañon	Vidal-Martínez *et al*., 2001; Mendoza-Franco *et al*., 2003
			Vieja maculicauda	Ron River	Vidal-Martínez *et al*., 2001; Mendoza-Franco *et al*., 2003
			Vieja maculicauda	Torsuani River	Mendoza-Palmero *et al*., 2017
	Sciadicleithrum nicaraguense		*Cribroheros alfari*	Loonku Creek, Bluefields	Vidal-Martínez *et al*., 2001; Mendoza-Franco *et al*., 2003
		A	*Cribroheros alfari*	Ron River	Vidal-Martínez *et al*., 2001; Mendoza-Franco *et al*., 2003
			Cribroheros alfari	Torsuani River	Vidal-Martínez *et al*., 2001; Mendoza-Franco *et al*., 2003
			Cribroheros longimanus	Crater lake Masaya	Santacruz *et al*., 2021
	Sciadicleithrum sp. 1	A	*Amatitlania nigrofasciata*	Crater lake Xiloá	Santacruz *et al*., 2021
	Sciadicleithrum sp. 2	A	*Cribroheros rostratus*	Lake Nicaragua	Santacruz *et al*., 2021
	Salsuginus neotropicalis	A	*Belonesox belizanus*	Ron River	Mendoza-Franco *et al*., 2003
	Palombitrema heteroancistrium	A	*Astyanax fasciatus*	Black Water River	Mendoza-Franco *et al*., 2003
			Astyanax fasciatus	El Padre River	Mendoza-Franco *et al*., 2003
Diplectanidae Monticelli, 1903	*Diplectanocotyla megalopis*	A	*Megalops atlanticus*	Loonku Creek, Bluefields	Mendoza-Franco *et al*., 2004
Hexabothriidae Price, 1942	*Erpocotyle carcharhini*	A	*Carcharhinus leucas*	Río San Juan, San Juan	Watson & Thorson, 1976
	Erpocotyle caribbensis	A	*Pristis perotteti*	Río Tipitapa, Los Cocos	Watson & Thorson, 1976
			Pristis perotteti	Río San Juan, San Juan	Watson & Thorson, 1976
	Pristonchotyle intermedia	A	*Pristis perotteti*	Río Tipitapa, Los Cocos	Watson & Thorson, 1976
			Pristis perotteti	Río San Juan, San Juan	Watson & Thorson, 1976
Acanthocephala					
Neoechinorhynchidae Ward, 1917	*Neoechinorhynchus costarricense* (=*Neoechinorhynchus golvani*)	A	*Amatitlania nigrofasciata*	Lake Nicaragua	Santacruz *et al*., 2021
			Amatitlania nigrofasciata	Crater lake Xiloá	Santacruz *et al*., 2021
			Amatitlania nigrofasciata	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus amarillo	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus astorquii	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus chancho	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus citrinellus	Lake Managua	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Asosca León	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Apoyeque	Santacruz *et al*., 2021

			Amphilophus citrinellus	Crater lake Masaya	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Nicaragua	Santacruz *et al*., 2021
			Amphilophus sagittae	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus xiloaensis	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus zaliosus	Crater lake Apoyo	Santacruz *et al*., 2021
			Cribroheros longimanus	Crater lake Xiloá	Santacruz *et al*., 2021
			Cribroheros alfari	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001b
			Herotilapia multispinosa	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001b
			Herotilapia multispinosa	Puente Chino	Aguirre-Macedo *et al*., 2001b
			Herotilapia multispinosa	Lake Nicaragua	Santacruz *et al*., 2021
			Hypsophrys nicaraguensis	Lake Nicaragua	Santacruz *et al*., 2021
			Hypsophrys nematopus	Lake Nicaragua	Santacruz *et al*., 2021
			Oreochromis sp.	Crater lake Asosca León	Santacruz *et al*., 2021
			Parachromis managuensis	Puente Chino	Aguirre-Macedo *et al*., 2001b
			Parachromis managuensis	Caño Negro	Aguirre-Macedo *et al*., 2001b
			Parachromis sp.	Lake Nicaragua	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Asosca León	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Apoyeque	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Xiloá	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Apoyo	Santacruz *et al*., 2021
Polymorphidae Meyer, 1931	*Polymorphus brevis*	L	*Amphilophus citrinellus*	Lake Nicaragua	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Nicaragua	Santacruz *et al*., 2021
			Hypsophrys nematopus	Lake Nicaragua	Santacruz *et al*., 2021
			Parachromis sp.	Lake Nicaragua	Santacruz *et al*., 2021
			Vieja sp.	Lake Nicaragua	Santacruz *et al*., 2021
Nematoda					
Anisakidae Railliet & Henry, 1912	*Contracaecum* sp.	L	*Cribroheros alfari*	Torsuani River	Aguirre-Macedo *et al*., 2001a
			Amatitlania nigrofasciata	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a
			Amphilophus amarillo	Crater lake Xiloá	González-Solís *et al*., 2006
			Amphilophus sagittae	Crater lake Xiloá	González-Solís *et al*., 2006
			Amphilophus xiloaensis	Crater lake Xiloá	González-Solís *et al*., 2006
			Astyanax fasciatus	Torsuani River	Aguirre-Macedo *et al*., 2001a
			Gobiomorus dormitor	Crater lake Xiloá	González-Solís *et al*., 2006
			Gobiomorus sp.	Crater lake Masaya	Santacruz *et al*., 2021
			Herotilapia multispinosa	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a
			Parachromis managuensis	Mahogany River	Aguirre-Macedo *et al*., 2001a
			Parachromis managuensis	Caño Negro	Aguirre-Macedo *et al*., 2001a
			Poecilia velifera	Torsuani River	Aguirre-Macedo *et al*., 2001a

			Vieja maculicauda	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a
	***Contracaecum* sp. 1**	L	*Amphilophus citrinellus*	Lake Nicaragua	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Nicaragua	Santacruz *et al*., 2021
			Amphilophus amarillo	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus astorquii	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus chancho	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus citrinellus	Lake Managua	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Asosca León	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Masaya	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Managua	Santacruz *et al*., 2021
			Amphilophus xiloaensis	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus xiloaensis	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus zaliosus	Crater lake Xiloá	Santacruz *et al*., 2021
			Archocentrus centrarchus	Lake Nicaragua	Santacruz *et al*., 2021
			Astyanax sp.	Lake Managua	**This study**
			Astyanax sp.	Lake Nicaragua	**This study**
			Cribroheros longimanus	Crater lake Masaya	Santacruz *et al*., 2021
			Cribroheros rostratus	Lake Nicaragua	Santacruz *et al*., 2021
			Cribroheros rostratus	Lake Managua	Santacruz *et al*., 2021
			Cribroheros rostratus	Crater lake Xiloá	Santacruz *et al*., 2021
			Hypsophrys nicaraguensis	Crater lake Xiloá	Santacruz *et al*., 2021
			Hypsophrys nematopus	Lake Nicaragua	Santacruz *et al*., 2021
			Hypsophrys nematopus	Crater lake Xiloá	Santacruz *et al*., 2021
			Hypsophrys nicaraguensis	Lake Nicaragua	Santacruz *et al*., 2021
			Parachromis sp.	Lake Nicaragua	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Asosca León	Santacruz *et al*., 2021
			Parachromis sp.	Lake Managua	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Xiloá	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Xiloá	Santacruz *et al*., 2021
			Vieja sp.	Lake Nicaragua	Santacruz *et al*., 2021
	Contracaecum sp. 2	L	*Amphilophus citrinellus*	Crater lake Asosca León	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Asosca León	Santacruz *et al*., 2021
	Contracaecum sp. 3	L	*Amphilophus citrinellus*	Crater lake Asosca León	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Asosca León	Santacruz *et al*., 2021
Atractidae Railliet, 1917	*Orientatractis chiapasensis*	A	*Tomocichla tuba*	Torsuani River	González-Solís & Moravec, 2004
Camallanidae Railliet & Henry, 1915	*Procamallanus neocaballeroi*	A	*Astyanax fasciatus*	Mahogany River	Aguirre-Macedo *et al*., 2001b
			Vieja maculicauda	Torsuani River	Aguirre-Macedo *et al*., 2001b

	Procamallanus barlowi (*=Procamallanus rebecae)*	A	*Cribroheros alfari*	Torsuani River	Aguirre-Macedo *et al*., 2001b
			Amphilophus citrinellus	Crater lake Apoyo	González-Solís *et al*., 2006
			Amatitlania nigrofasciata	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus astorquii	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus chancho	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus citrinellus	Lake Nicaragua	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Asosca León	Santacruz *et al*., 2021
			Amphilophus citrinellus	Lake Managua	Santacruz *et al*., 2021
			Amphilophus citrinellus	Crater lake Masaya	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Nicaragua	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Managua	Santacruz *et al*., 2021
			Amphilophus zaliosus	Crater lake Apoyo	Santacruz *et al*., 2021
			Cribroheros longimanus	Lake Nicaragua	Santacruz *et al*., 2021
			Cribroheros longimanus	Crater lake Masaya	Santacruz *et al*., 2021
			Cribroheros rostratus	Lake Nicaragua	Santacruz *et al*., 2021
			Herotilapia multispinosa	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001b
			Hypsophrys nematopus	Lake Nicaragua	Santacruz *et al*., 2021
			Hypsophrys nicaraguensis	Lake Nicaragua	Santacruz *et al*., 2021
			Parachromis managuensis	Crater lake Apoyo	González-Solís *et al*., 2006; Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Apoyeque	Santacruz *et al*., 2021
			Parachromis sp.	Lake Managua	Santacruz *et al*., 2021
			Vieja maculicauda	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001b
	Serpinema trispinosum	A	*Cribroheros alfari*	Puente Chino	Aguirre-Macedo *et al*., 2001a
			Herotilapia multispinosa	Puente Chino	Aguirre-Macedo *et al*., 2001a
			Parachromis managuensis	Puente Chino	Aguirre-Macedo *et al*., 2001a
Capillariidae Railliet, 1915	*Paracapillaria teixerafreitasi*	A	*Gobiomorus dormitor*	Crater lake Xiloá	González-Solís *et al*., 2006
Dioctophymatidae Railliet, 1915	*Eustrongylides* sp.	L	*Parachromis managuensis*	Mahogany River	Aguirre-Macedo *et al*., 2001a
Gnathostomatidae Railliet, 1895	*Spiroxys* sp.	L	*Cribroheros alfari*	Puente Chino	Aguirre-Macedo *et al*., 2001a
			Astyanax fasciatus	Puente Chino	Aguirre-Macedo *et al*., 2001a
			Herotilapia multispinosa	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a
			Parachromis managuensis	Caño Negro	Aguirre-Macedo *et al*., 2001a
			Parachromis managuensis	Mahogany River	Aguirre-Macedo *et al*., 2001a
			Parachromis managuensis	Puente Chino	Aguirre-Macedo *et al*., 2001a
			Vieja maculicauda	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a
Heterocheilidae Railliet & Henry, 1915	*Brevimulticaecum* sp.	L	*Astyanax fasciatus*	Torsuani River	Aguirre-Macedo *et al*., 2001a
			Rhamdia guatemalensis	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a
			Vieja maculicauda	Torsuani River	Aguirre-Macedo *et al*., 2001a

Kathlaniidae Lane, 1914	*Falcaustra* sp.	L	*Cribroheros alfari*	Puente Chino	Aguirre-Macedo *et al*., 2001a
			Amatitlania nigrofasciata	Puente Chino	Aguirre-Macedo *et al*., 2001a
			Herotilapia multispinosa	Mahogany River	Aguirre-Macedo *et al*., 2001a
			Herotilapia multispinosa	Loonku Creek, Bluefields	Aguirre-Macedo *et al*., 2001a
			Herotilapia multispinosa	Puente Chino	Aguirre-Macedo *et al*., 2001a
			Vieja maculicauda	Torsuani River	Aguirre-Macedo *et al*., 2001a
Raphidascarididae Hartwich, 1954	Acuariidae gen. sp.	L	*Amphilophus amarillo*	Crater lake Xiloá	González-Solís *et al*., 2006
	Goezia sp.	A	*Amphilophus sagittae*	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus xiloaensis	Crater lake Xiloá	González-Solís *et al*., 2006
			Amphilophus zaliosus	Crater lake Apoyo	Santacruz *et al*., 2021
	Hysterothylacium sp.	L	*Parachromis* sp.	Crater lake Xiloá	Santacruz *et al*., 2021
Rhabdochonidae Skrjabin, 1946	*Rhabdochona kidderi*	A	*Amphilophus amarillo*	Crater lake Xiloá	González-Solís *et al*., 2006
			Amphilophus citrinellus	Crater lake Apoyo	González-Solís *et al*., 2006
			Vieja maculicauda	Torsuani River	Aguirre-Macedo *et al*., 2001b
	Rhabdochona sp.	A	*Amphilophus citrinellus*	Lake Nicaragua	Santacruz *et al*., 2021
Spirocercidae Chitwood & Wehr, 1932	*Physocephalus* sp.	L	*Amphilophus labiatus*	Lake Nicaragua	Santacruz *et al*., 2021
Spiruridae Oerley, 1885	Spiruridae gen. sp.	L	*Gobiomorus dormitor*	Crater lake Xiloá	González-Solís *et al*., 2006
Hirudinea					
Piscicolidae Johnston, 1865	*Myzobdella* sp.	A	*Parachromis* sp.	Lake Nicaragua	Santacruz *et al*., 2021
Copepoda					
Ergasilidae Thorell, 1859	***Acusicola margulisae***	A	*Amphilophus citrinellus*	Crater lake Asosca León	Santacruz *et al*., 2020; Santacruz *et al*., 2021
			Oreochromis sp.	Crater lake Asosca León	Santacruz *et al*., 2020; Santacruz *et al*., 2021
			Poecilia sp.	Crater lake Asosca León	Santacruz *et al*., 2020; **This study**
	Acusicola sp.	A	*Amphilophus citrinellus*	Lake Nicaragua	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Nicaragua	Santacruz *et al*., 2021
	Ergasilidae gen. sp.	A	*Amphilophus citrinellus*	Lake Nicaragua	Santacruz *et al*., 2021; **this study**
			Amphilophus labiatus	Lake Nicaragua	Santacruz *et al*., 2021
			Amatitlania nigrofasciata	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus amarillo	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus astorquii	Crater lake Apoyo	Santacruz *et al*., 2021
			Amphilophus citrinellus	Lake Managua	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Managua	Santacruz *et al*., 2021
			Amphilophus sagittae	Crater lake Xiloá	Santacruz *et al*., 2021
			Amphilophus xiloaensis	Crater lake Xiloá	Santacruz *et al*., 2021
			Astyanax sp.	Lake Managua	**This study**

			Bramocharax sp.	Lake Nicaragua	**This study**
			Cribroheros longimanus	Crater lake Xiloá	Santacruz *et al*., 2021
			Cribroheros rostratus	Lake Managua	Santacruz *et al*., 2021
			Hypsophrys nematopus	Lake Nicaragua	Santacruz *et al*., 2021
			Hypsophrys nicaraguensis	Lake Nicaragua	Santacruz *et al*., 2021
			Hypsophrys nicaraguensis	Crater lake Xiloá	Santacruz *et al*., 2021
			Parachromis sp.	Lake Nicaragua	Santacruz *et al*., 2021
			Parachromis sp.	Lake Managua	Santacruz *et al*., 2021
			Parachromis sp.	Crater lake Xiloá	Santacruz *et al*., 2021
			Poecilia sp.	Lake Nicaragua	**This study**
			Poecilia sp.	Crater lake Xiloá	**This study**
			Vieja sp.	Lake Nicaragua	Santacruz *et al*., 2021
Lernaeidae Cobbold, 1879	***Lernaea cyprinacea***	A	*Astyanax* sp.	Lake Managua	**This study**
			Astyanax sp.	Lake Nicaragua	**This study**
			Bramocharax sp.	Lake Nicaragua	**This study**
			Hypsophrys nematopus	Lake Managua	Santacruz *et al*., 2021
	Lernaeidae gen. sp.	A	*Amphilophus citrinellus*	Lake Managua	Santacruz *et al*., 2021
			Amphilophus labiatus	Lake Managua	Santacruz *et al*., 2021
Branchiura					
Argulidae Leach, 1819	*Argulus* sp.	A	*Amphilophus citrinellus*	Crater lake Asosca León	Santacruz *et al*., 2021
			Amphilophus sagittae	Crater lake Xiloá	Santacruz *et al*., 2021
Oribatida					
---	**Oribatida gen. sp.**	A	*Parachromis* sp.	Crater lake Xiloá	Santacruz *et al*., 2021
			Poecilia sp.	Lake Nicaragua	**This study**

Supplementary Table S2. Host-parasite records for freshwater fishes in Nicaragua**.** The parasite group is indicated by T: trematoda, O: cestods, M: monogeneans, N: nematods, A: acanthocephalans, C: copepods, B: branchiurans, M: oribatids.

Host family	Host species	Parasite group	Parasite taxa
Bryconidae	*Brycon* sp.	T	*Saccocoelioides* sp.
Carcharhinidae	*Carcharhinus leucas*	M	*Dermophthirius maccallumi*
		M	*Heteronchocotyle leucas*
		M	*Erpocotyle carcharhini*

Characidae	*Astyanax aeneus*	T	*Prosthenhystera caballeroi*
		T	*Saccocoelioides tkachi*
	Astyanax fasciatus	T	*Auriculostoma astyanace*
		T	*Magnivitellum simplex*
		T	*Prosthenhystera obesa*
		T	*Uvulifer* sp.
		T	*Saccocoelioides* sp. 2
		T	*Genarchella astyanactys*
		T	*Ascocotyle tenuicollis*
		T	Proterodiplostomidae gen sp.
		O	Proteocephalidae gen. sp.
		M	Ancyrocephalinae sp.
		M	*Characithecium costaricensis*
		M	*Cleidodiscus strombicirrus*
		M	*Palombitrema heteroancistrium*
		N	*Contracaecum* sp.
		N	*Procamallanus neocaballeroi*
		N	*Spiroxys* sp.
		N	*Brevimulticaecum* sp.
	Astyanax sp.	T	*Austrodiplostomum compactum*
		T	*Genarchella astyanactys*
		T	*Centrocestus formosanus*
		M	*Characithecium costaricensis*
		N	*Contracaecum* sp. 1
		C	Ergasilidae gen. sp.
		C	*Lernaea cyprinacea*
	Bramocharax sp.	M	*Characithecium costaricensis*
		M	Dactylogyridae gen. sp.
		C	Ergasilidae gen. sp.
		C	*Lernaea cyprinacea*
	Roeboides guatemalensis	T	*Saccocoelioides* sp.
Cichlidae	*Alfaro cultratus*	T	*Uvulifer spinatus*
	Amatitlania nigrofasciata	T	*Posthodiplostomum minimum*
		T	*Posthodiplostomum* sp. 2
		T	*Uvulifer* sp.

	T	*Saccocoelioides orosiensis*
	T	*Crassicutis cichlasomae*
	M	*Sciadicleithrum bicuense*
	M	*Sciadicleithrum meekii*
	M	*Sciadicleithrum* sp. 1
	A	*Neoechinorhynchus costarricense*
	N	*Contracaecum* sp.
	N	*Procamallanus barlowi*
	N	*Falcaustra* sp.
	C	Ergasilidae gen. sp.
Amatitlania siquia	T	*Uvulifer* sp.
Amphilophus amarillo	T	*Oligogonotylus manteri*
	T	*Crassicutis cichlasomae*
	T	*Sciadicleithrum mexicanum*
	A	*Neoechinorhynchus costarricense*
	N	*Contracaecum* sp.
	N	*Contracaecum* sp. 1
	N	Acuariidae gen. sp.
	N	*Rhabdochona kidderi*
	C	Ergasilidae gen. sp.
Amphilophus astorquii	T	*Oligogonotylus manteri*
	T	*Saccocoelioides orosiensis*
	T	*Saccocoelioides* sp.
	T	*Crassicutis cichlasomae*
	M	*Sciadicleithrum mexicanum*
	A	*Neoechinorhynchus costarricense*
	N	*Contracaecum* sp. 1
	N	*Procamallanus barlowi*
	C	Ergasilidae gen. sp.
Amphilophus chancho	T	*Oligogonotylus manteri*
	T	*Saccocoelioides orosiensis*
	T	*Saccocoelioides* sp.
	T	*Crassicutis cichlasomae*
	M	*Sciadicleithrum mexicanum*
	A	*Neoechinorhynchus costarricense*
	N	*Contracaecum* sp. 1

	N	*Procamallanus barlowi*
Amphilophus citrinellus	T	*Oligogonotylus manteri*
	T	*Austrodiplostomum compactum*
	T	*Saccocoelioides* cf. *lamothei*
	T	*Saccocoelioides* sp.
	T	*Crassicutis cichlasomae*
	O	*Cichlidocestus janikae*
	O	*Schyzocotyle acheilognathi*
	M	*Sciadicleithrum mexicanum*
	A	*Neoechinorhynchus costarricense*
	A	*Polymorphus brevis*
	N	*Contracaecum* sp. 1
	N	*Contracaecum* sp. 2
	N	*Contracaecum* sp. 3
	N	*Procamallanus barlowi*
	N	*Rhabdochona kidderi*
	N	*Rhabdochona* sp.
	C	*Acusicola margulisae*
	C	*Acusicola* sp.
	C	Ergasilidae gen. sp.
	C	Lernaeidae gen. sp.
	B	*Argulus* sp.
Amphilophus flaveolus	T	*Saccocoelioides* sp.
	T	*Crassicutis cichlasomae*
	M	*Sciadicleithrum mexicanum*
Amphilophus globosus	T	*Oligogonotylus manteri*
	T	*Saccocoelioides* sp.
	T	*Crassicutis cichlasomae*
Amphilophus labiatus	T	*Oligogonotylus manteri*
	T	*Ascocotyle pindoramensis*
	T	*Crassicutis cichlasomae*
	A	*Neoechinorhynchus costarricense*
	A	*Polymorphus brevis*
	N	*Contracaecum* sp. 1
	N	*Procamallanus barlowi*
	N	*Physocephalus* sp.

	C	*Acusicola* sp.
	C	Ergasilidae gen. sp.
	C	Lernaeidae gen. sp.
Amphilophus sagittae	T	*Oligogonotylus manteri*
	T	*Saccocoelioides orosiensis*
	T	*Saccocoelioides* sp.
	T	*Crassicutis cichlasomae*
	M	*Sciadicleithrum mexicanum*
	A	*Neoechinorhynchus costarricense*
	N	*Contracaecum* sp.
	N	*Goezia* sp.
	C	Ergasilidae gen. sp.
	B	*Argulus* sp.
Amphilophus xiloaensis	T	*Oligogonotylus manteri*
	T	*Saccocoelioides orosiensis*
	T	*Crassicutis cichlasomae*
	M	*Sciadicleithrum mexicanum*
	A	*Neoechinorhynchus costarricense*
	N	*Contracaecum* sp.
	N	*Contracaecum* sp. 1
	N	*Goezia* sp.
	C	Ergasilidae gen. sp.
Amphilophus zaliosus	T	*Oligogonotylus manteri*
	T	*Saccocoelioides orosiensis*
	T	*Saccocoelioides* sp.
	T	*Crassicutis cichlasomae*
	M	*Sciadicleithrum mexicanum*
	A	*Neoechinorhynchus costarricense*
	N	*Contracaecum* sp. 1
	N	*Procamallanus barlowi*
	N	*Goezia* sp.
Archocentrus centrarchus	T	*Uvulifer* sp.
	T	*Phyllodistomum romualdae*
	T	*Crassicutis cichlasomae*
	N	*Contracaecum* sp. 1
Cribroheros alfari	T	*Austrodiplostomum compactum*

	T	*Uvulifer* sp.
	T	Heterophyidae gen. sp.
	M	*Sciadicleithrum nicaraguense*
	A	*Neoechinorhynchus costarricense*
	N	*Contracaecum* sp.
	N	*Procamallanus barlowi*
	N	*Serpinema trispinosum*
	N	*Spiroxys* sp.
	N	*Falcaustra* sp.
Cribroheros longimanus	T	*Oligogonotylus manteri*
	T	*Uvulifer* sp.
	T	*Crassicutis cichlasomae*
	M	*Sciadicleithrum nicaraguense*
	A	*Neoechinorhynchus costarricense*
	N	*Contracaecum* sp. 1
	N	*Procamallanus barlowi*
	C	Ergasilidae gen. sp.
Cribroheros rostratus	T	*Oligogonotylus manteri*
	T	*Austrodiplostomum compactum*
	T	*Crassicutis cichlasomae*
	M	*Sciadicleithrum* sp. 2
	N	*Contracaecum* sp. 1
	N	*Procamallanus barlowi*
	C	Ergasilidae gen. sp.
Cryptoheros spilurus	T	*Crassicutis cichlasomae*
Herotilapia multispinosa	T	*Posthodiplostomum minimum*
	T	*Uvulifer* sp.
	T	Heterophyidae gen. sp.
	T	Proterodiplostomidae gen sp.
	O	Proteocephalidae gen. sp.
	M	*Gussevia herotilapiae*
	A	*Neoechinorhynchus costarricense*
	N	*Contracaecum* sp.
	N	*Procamallanus barlowi*
	N	*Serpinema trispinosum*
	N	*Spiroxys* sp.

	N	*Falcaustra* sp.
Hypsophrys nematopus	T	*Ithyoclinostomum yamaguti*
	T	*Oligogonotylus manteri*
	T	*Posthodiplostomum* sp. 1
	T	*Posthodiplostomum* sp. 2
	T	*Saccocoelioides* cf. *lamothei*
	T	*Saccocoelioides orosiensis*
	T	*Saccocoelioides* sp.
	T	*Crassicutis cichlasomae*
	T	Strigeidae gen sp.
	A	*Neoechinorhynchus costarricense*
	A	*Polymorphus brevis*
	N	*Contracaecum* sp. 1
	N	*Procamallanus barlowi*
	C	Ergasilidae gen. sp.
	C	*Lernaea cyprinacea*
Hypsophrys nicaraguensis	T	*Ithyoclinostomum yamaguti*
	T	*Oligogonotylus manteri*
	T	*Saccocoelioides orosiensis*
	T	*Crassicutis cichlasomae*
	O	*Cichlidocestus janikae*
	A	*Neoechinorhynchus costarricense*
	N	*Contracaecum* sp. 1
	N	*Procamallanus barlowi*
	C	Ergasilidae gen. sp.
Hypsophrys sp.	T	*Uvulifer* sp.
Mayaheros urophthalmus	M	*Sciadicleithrum mexicanum*
Oreochromis sp.	T	*Saccocoelioides* cf. *lamothei*
	T	*Saccocoelioides orosiensis*
	T	*Saccocoelioides* sp.
	T	Heterophyidae gen. sp.
	M	*Cichlidogyrus sclerosus*
	A	*Neoechinorhynchus costarricense*
	C	*Acusicola margulisae*
Parachromis dovii	M	*Sciadicleithrum mexicanum*

Parachromis managuensis	T	*Ithyoclinostomum yamaguti*
	T	*Oligogonotylus manteri*
	T	*Posthodiplostomum minimum*
	T	Heterophyidae gen. sp.
	T	*Crassicutis cichlasomae*
	T	*Cladocystis trifolium*
	O	Proteocephalidae gen. sp.
	M	*Sciadicleithrum mexicanum*
	A	*Neoechinorhynchus costarricense*
	N	*Contracaecum* sp.
	N	*Procamallanus barlowi*
	N	*Serpinema trispinosum*
	N	*Eustrongylides* sp.
	N	*Spiroxys* sp.
Parachromis sp.	T	*Ithyoclinostomum yamaguti*
	T	Cryptogonimidae gen. sp.
	T	*Oligogonotylus manteri*
	T	*Austrodiplostomum compactum*
	T	*Saccocoelioides* sp.
	T	*Ascocotyle pindoramensis*
	T	*Crassicutis cichlasomae*
	O	*Schyzocotyle acheilognathi*
	M	*Sciadicleithrum mexicanum*
	A	*Neoechinorhynchus costarricense*
	A	*Polymorphus brevis*
	N	*Contracaecum* sp. 1
	N	*Contracaecum* sp. 3
	N	*Procamallanus barlowi*
	N	*Hysterothylacium* sp.
	H	*Myzobdella* sp.
	C	Ergasilidae gen. sp.
	M	Oribatida gen. sp.
Tomocichla tuba	M	*Sciadicleithrum mexicanum*
	N	*Orientatractis chiapasensis*
Vieja maculicauda	T	*Acanthostomum minimum*

		T	*Oligogonotylus manteri*
		T	*Posthodiplostomum minimum*
		T	*Uvulifer* sp.
		T	*Saccocoelioides cichlidorum*
		T	*Saccocoelioides* sp. 1
		T	*Saccocoelioides* sp. 2
		T	*Ascocotyle nana*
		T	*Ascocotyle tenuicollis*
		T	*Crassicutis cichlasomae*
		M	*Sciadicleithrum bravohollisae*
		M	*Sciadicleithrum maculicaudae*
		M	*Sciadicleithrum mexicanum*
		N	*Contracaecum* sp.
		N	*Procamallanus neocaballeroi*
		N	*Procamallanus barlowi*
		N	*Spiroxys* sp.
		N	*Brevimulticaecum* sp.
		N	*Falcaustra* sp.
		N	*Rhabdochona kidderi*
	Vieja sp.	A	*Polymorphus brevis*
		N	*Contracaecum* sp. 1
		C	Ergasilidae gen. sp.
Eleotridae	*Gobiomorus dormitor*	N	*Contracaecum* sp.
		N	*Paracapillaria teixerafreitasi*
		N	Spiruridae gen. sp.
	Gobiomorus sp.	N	*Contracaecum* sp.
Haemulidae	*Pomadasys croco*	T	*Neochasmus ackerti*
Heptateridae	*Rhamdia guatemalensis*	N	*Brevimulticaecum* sp.
	Rhamdia managuensis	T	*Acanthostomum gnerii*
		T	*Genarchella thorsoni*
		T	Proterodiplostomidae gen sp.
	Rhamdia nicaraguensis	T	*Acanthostomum astorquii*
		T	*Acanthostomum gnerii*
		T	*Phyllodistomum scotti*
		M	*Aphanoblastella travassosi*
		M	*Cleidodiscus chavarriai*

	Rhamdia sp.	T	*Clinostomum* sp.
		O	Proteocephalidae gen. sp.
Lepisosteidae	*Atractosteus tropicus*	T	*Perezitrema* sp.
		O	*Proteocephalus gaspari*
Megalopidae	*Megalops atlanticus*	M	*Diplectanocotyla megalopis*
Poeciliidae	*Belonesox belizanus*	M	*Salsuginus neotropicalis*
	Poecilia mexicana	T	*Uvulifer spinatus*
	Poecilia sp.	T	*Posthodiplostomum* sp. 1
		T	*Saccocoelioides* cf. *lamothei*
		T	*Saccocoelioides orosiensis*
		T	*Saccocoelioides* sp.
		O	Cyclophylidea gen. sp.
		C	*Acusicola margulisae*
		C	Ergasilidae gen. sp.
		M	Oribatida gen. sp.
	Poecilia velifera	T	Cryptogonimidae gen. sp.
		T	*Posthodiplostomum minimum*
		T	*Saccocoelioides sogandaresi*
		T	*Ascocotyle diminuta*
		T	*Ascocotyle mollienisicola*
		T	*Ascocotyle tenuicollis*
		N	*Contracaecum* sp.
Pristidae	*Pristis perotteti*	M	*Erpocotyle caribbensis*
		M	*Pristonchotyle intermedia*

Supplemetary Table S3. Host-parasite list for fish in freshwater systems in Costa Rica.

Family	Parasite	Host	Locality	Reference
Trematoda				
Allocreadiidae Looss, 1902	*Auriculostoma astyanace*	*Astyanax aeneus*	Animas River, Guanacaste	Curran *et al*., 2011
	Creptotrematina aguirrepequenoi	*Astyanax aeneus*	Tempisque River, Guanacaste	Curran *et al*., 2011; Pérez-Ponce de León *et al*., 2016
	Paracreptotrema blancoi	*Priapichthys annectens*	Orosí River	Choudhury *et al.,* 2006
		Priapichthys annectens	Guanacaste	Sandund *et al*., 2010

		Priapichthys annectens	Quebrada Plata	Pérez-Ponce de León *et al.*, 2016
	Paracreptotrema mendezi	*Brachyrhapis olomina*	Tempisque River, Guanacaste	Sandund *et al.*, 2010
Acanthocolpidae Lühe, 1906	*Acanthostomum minimum (=Stunkardiella minima)*	*Rhamdia nicaraguense*	Guanacaste	Sandund *et al.*, 2010
		Rhamdia rogersi	Paso Ancho, San José	Rodríguez-Ortíz *et al.*, 2004
Callodistomidae Odhner, 1910	*Prosthenhystera obesa*	*Astyanax aeneus*	Guanacaste	Sandund *et al.*, 2010
	Prosthenhystera caballeroi	*Astyanax aeneus*	Tempisque River, Guanacaste	Tkach *et al.*, 2015
Clinostomidae Lühe, 1901	*Clinostomatid*	*Poecilia gillii*	Quebrada	Chandler *et al.*, 1995
		Agonostomus monticola	Guanacaste	Sandund *et al.*, 2010
		Archocentrus nigrofasciatus	Guanacaste	Sandund *et al.*, 2010
		Astatheros alfari	Guanacaste	Sandund *et al.*, 2010
		Gymnotus cylindricus	Guanacaste	Sandund *et al.*, 2010
		Rhamdia rogersi	Guanacaste	Sandund *et al.*, 2010
		Sicydium salvini	Guanacaste	Sandund *et al.*, 2010
	Ithyoclinostomum yamagutii	*Cribroheros longimanus*	Irigaray River	Briosio-Aguilar *et al.*, 2019
		Parachromis managuense	Irigaray River	Briosio-Aguilar *et al.*, 2019
		Cribroheros alfari	Orosí River, Pitahaya	Briosio-Aguilar *et al.*, 2019
Crytogonimidae Ward, 1917	*Pseudocaecincola* sp.	*Parachromis dovii*	Guanacaste	Sandund *et al.*, 2010
	Oligogonotylus manteri	*Parachromis dovii*	Guanacaste	Sandund *et al.*, 2010
Cyathocotylidae Mühling, 1898	*Mesostephanus* sp.	*Agonostomus monticola*	Guanacaste	Sandund *et al.*, 2010
Derogenidae Nicoll, 1910	*Genarchella* sp.	*Parachromis dovii*	Guanacaste	Sandund *et al.*, 2010
Diplostomatidae Poirier, 1886	*Austrodiplostomum compactum*	*Astatheros alfari*	Río Las Vueltas	Sereno-Uribe *et al.*, 2019
		Archocentrus siquia	Río Las Vueltas	Sereno-Uribe *et al.*, 2019
		Amphilophus alfari	Río Irigaray	Sereno-Uribe *et al.*, 2019
		Hypsophrys nicaraguensis	Lago el Arenal	Sereno-Uribe *et al.*, 2019
	Diplostomum	*Poecilia gillii*	Quebrada	Chandler *et al.*, 1995
Gorgoderidae Looss, 1899	*Phyllodistomum romualdae*	*Cribroheros alfari*	Río Las Animas	Pinacho-Pinacho *et al.*, 2021
Haploporidae Nicoll, 1914	*Saccocoelioides cichlidorum*	*Archocentrus nigrofasciatus*	Río Las Animas	Andrade-Gómez *et al.*, 2017
		Astatheros alfari	Río Las Animas	Andrade-Gómez *et al.*, 2017
		Archocentrus siquia	Río Las Animas	Andrade-Gómez *et al.*, 2017
		Hypsophrys nematopus	Orosí River	Andrade-Gómez *et al.*, 2017
		Archocentrus siquia	Irigaray River	Andrade-Gómez *et al.*, 2017
	Saccocoelioides lamothei	*Poecilia gillii*	Rio Tempisque	Curran *et al.,* 2018
		Poecilia gillii	Rio Mico	González-García *et al.*, 2020
		Sicydium salvini	Río Ciruelas	González-García *et al.*, 2020

		Poecilia gillii	Río Ciruelas	González-García *et al.*, 2020
	Saccocoelioides orosiensis	*Poecilia gillii*	Río Ciruelas	Andrade-Gómez *et al.,* 2019
	Saccocoelioides sogandaresi	*Poecilia gillii*	Irigaray River	Andrade-Gómez *et al*., 2017
		Poecilia gillii	Río Ciruelas	Andrade-Gómez *et al*., 2017
		Poecilia gillii	Río Las Vueltas	Andrade-Gómez *et al*., 2017
		Poecilia gillii	Quebrada Puercos	Andrade-Gómez *et al*., 2017
		Poecilia gillii	Centeno	Andrade-Gómez *et al*., 2017
		Poecilia gillii	Guanacaste	Sandund *et al.,* 2010
		Poecilia mexicana	Playa Grande (Puerto viejo)	Andrade-Gómez *et al*., 2017
	Saccocoelioides sp.	*Astyanax aeneus*	Guanacaste	Sandund *et al.,* 2010
		Brycon guatemalensis	Guanacaste	Sandund *et al.,* 2010
		Hypsophrys nicaraguensis	Guanacaste	Sandund *et al.,* 2010
	Saccocoelioides sp. 1	*Bryconamericus scleroparius*	Guanacaste	Sandund *et al.,* 2010
	Saccocoelioides sp. 3	*Archocentrus nigrofasciatus*	Guanacaste	Sandund *et al.,* 2010
		Hypsophrys nematopus	Guanacaste	Sandund *et al.,* 2010
	Saccocoelioides tkachi	*Astyanax aeneus*	Río Tempisque	Curran *et al*., 2018
Heterophyidae Leiper, 1909	*Centrocestus formosanus*	*Oreochromis niloticus*	--	Arguedas et al., 2010
Lepocreadiidae Odhner, 1905	*Crassicutis cichlasomae*	*Archocentrus nigrofasciatus*	Guanacaste	Sandund *et al.,* 2010
		Astatheros alfari	Guanacaste	Sandund *et al.,* 2010
		Cribroheros longimanus	Guanacaste	Sandund *et al.,* 2010
		Hypsophrys nicaraguensis	Guanacaste	Sandund *et al.,* 2010
		Parachromis dovii	Guanacaste	Sandund *et al.,* 2010
		Rhamdia nicaraguense	Guanacaste	Sandund *et al.,* 2010
Macroderoididae McMullen, 1937	*Wallinia chavarriae*	*Astyanax aeneus*	Quebrada Limonal	Choudhury *et al*., 2002
		Astyanax aeneus	Río Sapoa	Choudhury *et al*., 2002
		Astyanax aeneus	Guanacaste	Sandlund et al., 2010
		Bryconamericus scleroparius	Quebrada Limonal	Choudhury *et al*., 2002
		Bryconamericus scleroparius	Río Sapoa	Choudhury *et al*., 2002
		Bryconamericus scleroparius	Guanacaste	Sandund *et al.,* 2010
Strigeidae Railliet, 1919	*Neascus*	*Poecilia gillii*	Quebrada	Chandler *et al.,* 1995
	Strigeidae gen. sp.	*Rhamdia rogersi*	Guanacaste	Sandund *et al.,* 2010
Strigeoidea	*Uvulifer* sp. lineage 2	*Hypsophrys nematopus*	Orosí River	López-Jiménez *et al*., 2018
-----	Digenea gen. sp.	*Archocentrus nigrofasciatus*	Guanacaste	Sandund *et al.,* 2010
		Arius guatemalensis	Guanacaste	Sandund *et al.,* 2010
		Arius seemani	Guanacaste	Sandund *et al.,* 2010

		Astyanax aeneus	Guanacaste	Sandund *et al.*, 2010
		Brachyrhapis olomina	Guanacaste	Sandund *et al.*, 2010
		Bryconamericus scleroparius	Guanacaste	Sandund *et al.*, 2010
		Eleotris picta	Guanacaste	Sandund *et al.*, 2010
		Gobiomorus dormitor	Guanacaste	Sandund *et al.*, 2010
		Hypsophrys nematopus	Guanacaste	Sandund *et al.*, 2010
		Poecilia gillii	Guanacaste	Sandund *et al.*, 2010
		Priapichthys annectens	Guanacaste	Sandund *et al.*, 2010
		Rhamdia nicaraguense	Guanacaste	Sandund *et al.*, 2010
		Rhamdia rogersi	Guanacaste	Sandund *et al.*, 2010
		Rivulus isthmensis	Guanacaste	Sandund *et al.*, 2010
Cestoda				
Proteocephalidae La Rue, 1911	*Cichlidocestus janikae*	*Hypsophrys nicaraguensis*	Lake Arenal	de Chambrier *et al*., 2017
	Proteocephalidea gen. sp.	*Agonostomus monticola*	Guanacaste	Sandund *et al.*, 2010
		Astatheros alfari	Guanacaste	Sandund *et al.*, 2010
		Awaous transandeanus	Guanacaste	Sandund *et al.*, 2010
		Eleotris picta	Guanacaste	Sandund *et al.*, 2010
		Parachromis dovii	Guanacaste	Sandund *et al.*, 2010
		Synbranchus marmoratus	Guanacaste	Sandund *et al.*, 2010
Monogenea				
	Monogenea gen. sp.	*Agonostomus monticola*	Guanacaste	Sandund *et al.*, 2010
		Astyanax aeneus	Guanacaste	Sandund *et al.*, 2010
		Brachyrhapis olomina	Guanacaste	Sandund *et al.*, 2010
		Bryconamericus scleroparius	Guanacaste	Sandund *et al.*, 2010
		Parachromis dovii	Guanacaste	Sandund *et al.*, 2010
		Parachromis managuense	Guanacaste	Sandund *et al.*, 2010
		Poecilia gillii	Guanacaste	Sandund *et al.*, 2010
		Rhamdia rogersi	Guanacaste	Sandund *et al.*, 2010
Acanthocephala				
-----	Acanthocephala gen. sp.	*Eleotris picta*	Guanacaste	Sandund *et al.*, 2010
Neoechinorhynchidae Ward, 1917	*Neoechinorhynchus* Lineage 7 /*N. mamesi*	*Dormitator maculatus*	Playa Grande	Pinacho-Pinacho *et al.*, 2015
		Dormitator latifrons	Quebrada Ganados	Pinacho-Pinacho *et al.*, 2015
	Neoechinorhynchus costarricense	*Parachromis managuense*	Lago Jalapa	Pinacho-Pinacho *et al.,* 2018
		Parachromis loisellei	Lago Jalapa	Pinacho-Pinacho *et al.,* 2018
		Cribroheros longimanus	Lago Jalapa	Pinacho-Pinacho *et al.,* 2018

		Heterotilapia multiespinosa	Lago Jalapa	Pinacho-Pinacho *et al.,* 2018
		Archocentrus centrarchus	Lago Jalapa	Pinacho-Pinacho *et al.,* 2018
		Amatitlania nigrofasciata	Quebrada Puercos	Pinacho-Pinacho *et al.,* 2018
		Amatitlania siquia	--	Pinacho-Pinacho *et al.*, 2018b
		Cribroheros alfari	--	Pinacho-Pinacho *et al.*, 2018b
		Parachromis managuense	Guanacaste	Sandund *et al.,* 2010
Nematoda				
Anisakidae Railliet & Henry, 1912	*Contracaecum* sp.	*Agonostomus monticola*	Guanacaste	Sandund *et al.,* 2010
		Arius seemani	Guanacaste	Sandund *et al.,* 2010
		Astatheros alfari	Guanacaste	Sandund *et al.,* 2010
		Astyanax aeneus	Guanacaste	Sandund *et al.,* 2010
		Cribroheros longimanus	Guanacaste	Sandund *et al.,* 2010
		Gobiomorus dormitor	Guanacaste	Sandund *et al.,* 2010
		Parachromis managuense	Guanacaste	Sandund *et al.,* 2010
		Phallichthys amates	Guanacaste	Sandund *et al.,* 2010
		Rhamdia nicaraguense	Guanacaste	Sandund *et al.,* 2010
		Rhamdia rogersi	Guanacaste	Sandund *et al.,* 2010
		Rivulus isthmensis	Guanacaste	Sandund *et al.,* 2010
		Synbranchus marmoratus	Guanacaste	Sandund *et al.,* 2010
Atractidae Railliet, 1917	*Rondonia* cf. *rondoni*	*Archocentrus nigrofasciatus*	Guanacaste	Sandund *et al.,* 2010
		Hypsophrys nematopus	Guanacaste	Sandund *et al.,* 2010
Camallanidae Railliet & Henry, 1915	*Procamallanus neocaballeroi*	*Astyanax aeneus*	Guanacaste	Sandund *et al.,* 2010
	Procamallanus barlowi	*Amatitlania nigrofasciata*	Río Las Animas	Santacruz *et al.*, 2021
		Archocentrus nigrofasciatus	Guanacaste	Sandund *et al.,* 2010
		Astatheros alfari	Guanacaste	Sandund *et al.,* 2010
		Cribroheros longimanus	Río Irigaray	Santacruz *et al.*, 2021
		Hypsophrys nematopus	Guanacaste	Sandund *et al.,* 2010
		Parachromis dovii	Guanacaste	Sandund *et al.,* 2010
	Procamallanus sp.	*Agonostomus monticola*	Guanacaste	Sandund *et al.,* 2010
		Astatheros alfari	Guanacaste	Sandund *et al.,* 2010
		Awaous transandeanus	Guanacaste	Sandund *et al.,* 2010
		Brachyrhapis olomina	Guanacaste	Sandund *et al.,* 2010
		Rhamdia guatemalensis	Guanacaste	Sandund *et al.,* 2010
		Rhamdia nicaraguense	Guanacaste	Sandund *et al.,* 2010
Capillariidae Railliet, 1915	Capilariidae gen. sp.	*Gobiomorus dormitor*	Guanacaste	Sandund *et al.,* 2010
		Rhamdia nicaraguense	Guanacaste	Sandund *et al.,* 2010

		Rhamdia rogersi	Guanacaste	Sandund *et al.*, 2010
Cucullanidae Cobbold, 1864	*Cucullanus costaricensis*	*Bagre pinnimaculatus*	Río Tempisque	López-Caballero *et al.*, 2009
	Cucullanidae gen. sp.	*Arius seemani*	Guanacaste	Sandund *et al.*, 2010
	Cucullanus pimelodellae	*Rhamdia guatemalensis*	Guanacaste	Sandund *et al.*, 2010
		Rhamdia nicaraguense	Guanacaste	Sandund *et al.*, 2010
Cystidicolidae Skrjabin, 1946	*Spinitectus agonostomi*	*Agonostomus monticola*	Guanacaste	Sandund *et al.*, 2010
	Spinitectus mexicanus	*Priapichthys annectens*	Guanacaste	Sandund *et al.*, 2010
	Spinitectus sp.	*Alfaro cultratus*	Guanacaste	Sandund *et al.*, 2010
		Rivulus isthmensis	Guanacaste	Sandund *et al.*, 2010
Raphidascarididae Hartwich, 1954	*Hysterothylacium* sp.	*Astatheros alfari*	Guanacaste	Sandund *et al.*, 2010
Rhabdochonidae Skrjabin, 1946	*Rhabdochona kidderi*	*Archocentrus nigrofasciatus*	Guanacaste	Sandund *et al.*, 2010
		Astatheros alfari	Guanacaste	Sandund *et al.*, 2010
		Cribroheros longimanus	Guanacaste	Sandund *et al.*, 2010
		Hypsophrys nematopus	Guanacaste	Sandund *et al.*, 2010
		Parachromis dovii	Guanacaste	Sandund *et al.*, 2010
	Rhabdochona acuminata	*Bryconamericus scleroparius*	Guanacaste	Sandund *et al.*, 2010
	Rhabdochona cf. *cubensis*	*Rivulus isthmensis*	Guanacaste	Sandund *et al.*, 2010
	Rhabdochona sp.	*Astatheros alfari*	Guanacaste	Sandund *et al.*, 2010
		Parachromis dovii	Guanacaste	Sandund *et al.*, 2010
		Rhamdia rogersi	Guanacaste	Sandund *et al.*, 2010
	Rhabdochonidae gen. sp.	*Alfaro cultratus*	Guanacaste	Sandund *et al.*, 2010
		Archocentrus nigrofasciatus	Guanacaste	Sandund *et al.*, 2010
		Eleotris picta	Guanacaste	Sandund *et al.*, 2010
		Gobiomorus maculatus	Guanacaste	Sandund *et al.*, 2010
		Priapichthys annectens	Guanacaste	Sandund *et al.*, 2010
Spiruroidea Oerley, 1885	*Spiroxys* sp.	*Astatheros alfari*	Guanacaste	Sandund *et al.*, 2010
Spiruridae Oerley, 1885	Spirurida gen. sp.	*Alfaro cultratus*	Guanacaste	Sandund *et al.*, 2010
		Priapichthys annectens	Guanacaste	Sandund *et al.*, 2010
		Rhamdia rogersi	Guanacaste	Sandund *et al.*, 2010
-----	Nematoda gen. sp.	*Agonostomus monticola*	Guanacaste	Sandund *et al.*, 2010
		Alfaro cultratus	Guanacaste	Sandund *et al.*, 2010
		Archocentrus nigrofasciatus	Guanacaste	Sandund *et al.*, 2010
		Bryconamericus scleroparius	Guanacaste	Sandund *et al.*, 2010
		Cribroheros longimanus	Guanacaste	Sandund *et al.*, 2010
		Eleotris picta	Guanacaste	Sandund *et al.*, 2010
		Parachromis managuense	Guanacaste	Sandund *et al.*, 2010
		Roeboides bouchellei	Guanacaste	Sandund *et al.*, 2010

Copepoda				
Ergasilidae Thorell, 1859	Ergasilidae gen. sp.	*Astyanax aeneus*	Guanacaste	Sandund *et al.,* 2010
		Bryconamericus scleroparius	Guanacaste	Sandund *et al.,* 2010
		Hypsophrys nematopus	Guanacaste	Sandund *et al.,* 2010
		Poecilia gillii	Guanacaste	Sandund *et al.,* 2010

Supplemetary table S4. Host-parasite list for fish in freshwater systems in Panamá.

Family	Parasite	Host	Locality	Reference
Trematoda				
Acanthostomatidae Poche, 1926	*Acanthostomum minimum* (=*Stunkardiella minima*)	*Vieja maculicauda*	Panama Canal	Roche *et al*., 2010
	Atrophacaecum astorquii	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
		Oreochromis niloticus	Panama Canal	Roche *et al.,* 2010
	Pelaezia loosi	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
		Oreochromis niloticus	Panama Canal	Roche *et al.,* 2010
Allocreadiidae Losos, 1902	*Creptotrema* sp.	*Pimelodella chagresi*	Río Frijolito	Choudhury *et al*., 2017
	Creptotrematina sp.	*Bryconamericus emperador*	Quebrada Juan Grande	Choudhury *et al*., 2017
	Paracreptotrema mendezi (= Fellodistomum mendezi)	*Brachyrhaphis episcopi*	Gatun Lake; Barro Colorado Island	Sogandares-Bernal, 1955; Choudhury *et al*., 2017
	Wallinia chavarriae	*Gephyrocharax atricaudatus*	Río Frijolito	Choudhury *et al*., 2017
Callodistomidae Poche, 1926	*Prosthenhystera obesa*	*Bryconamericus emperador*	Río Frijolito	Choudhury *et al*., 2017
Clinostomidae Lühe, 1901	*Clinostomum complanatum*	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
Crytogonimidae Ward, 1917	*Acanthostomum gnerii*	*Pimelodella chagresi*	Río Frijolito	Choudhury *et al*., 2017
	Oligogonotylus manteri	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
Derogenidae Nicoll, 1910	*Genarchella* sp. *(= Halipegus genarchellus)*	*Roeboides guatemalensis*	Quebrada Juan Grande	Sogandares-Bernal, 1955; Choudhury *et al*., 2017
		Hoplias malabaricus	Quebrada Juan Grande	Choudhury *et al*., 2017
		Vieja maculicauda	Panama Canal	Roche *et al.,* 2010
Diplostomatidae Poirier, 1886	*Diplostomum compactum*	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
		Oreochromis niloticus	Panama Canal	Roche *et al.,* 2010
	Posthodiplostomum sp.	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
Fellodistomatidae Nicoll, 1913	*Fellodistomum mendez*	*Brachyrhaphis episcopi*	Gatun Lake	Sogandares-Bernal, 1955
Gorgoderidae Looss, 1899	*Phyllodistomum* sp.	*Pimelodella chagresi*	Río Frijolito	Choudhury *et al*., 2017
		Hoplias microlepis	Quebrada Juan Grande	Choudhury *et al*., 2017
Haploporidae Nicoll, 1914	*Saccocoelioides cichlidorum (=Culuwiya cichlidorum)*	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
		Oreochromis niloticus	Panama Canal	Roche *et al.,* 2010
Heterophyidae Leiper, 1909	*Ascocotyle sp.*	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010

Hemuridae	*Halipegus genarchellus*	*Roeboides guatemalensis*	Gatun Lake	Sogandares-Bernal, 1955
Opisthorchiidae Losss, 1899	*Cladocystis trifolium*	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
Proterodiplostomidae Dubois, 1936	*Proterodiplostomidae* sp. 1	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
	Proterodiplostomidae sp. 2	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
Cestoda				
Bothiocephalidae	*Schyzocotyle acheilognathi*	*Aequidens coeruleopunctatus*	Chagres River, Panama	Choudhury *et al*., 2013
		Aequidens coeruleopunctatus	Quebrada Juan Grande	Choudhury *et al*., 2017
		Cryptoheros panamensis	Chagres River, Panama	Choudhury *et al.,* 2013
			Quebrada Juan Grande	Choudhury *et al*., 2017
Monogenea				
Dactylogyridae	*Aphanoblastella chagresii*	*Pimelodella chagresi*	Río Frijolito	Mendoza-Franco *et al.,* 2007
	Aphanoblastella travassosi	*Rhamdia quelen*	Lago Alajuela	Mendoza-Franco *et al*., 2007
	Aristocleidus hastatus	*Eugerres brasilianus*	Lake Gatun	Mendoza-Franco *et al*., 2009b
	Characithecium costaricensis	*Astyanax aeneus*	Nigua River	Mendoza-Franco *et al*., 2009
	Cichlidogyrus dossoui	*Oreochromis niloticus*	Panama Canal	Roche *et al.,* 2010
	Cichlidogyrus sp. 1	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
	Cichlidogyrus sp. 2	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
	Diaphorocleidus kabatai	*Astyanax aeneus*	Nigua, Caldera	Mendoza-Franco *et al*., 2009
		Astyanax aeneus	Chiriquicito Rivers	Mendoza-Franco *et al*., 2009
	Diaphorocleidus orthodusus	*Astyanax orthodus*	Nigua River	Mendoza-Franco *et al*., 2009
	Diaphorocleidus petrosusi	*Brycon petrosus*	Río Frijolito	Mendoza-Franco *et al*., 2007
	Diplectanum gatunense	*Eugerres brasilianus*	Gatun Lake	Mendoza-Franco *et al*., 2008
	Gussevia asota	*Astronotus ocellatus*	Lago Gatun	Mendoza-Franco *et al*., 2007
	Neotetraonchus vegrandis	*Ariopsis guatemalensis*	Puerto Mutis, Gulf of Montijo	Kritsky *et al.*, 2009
	Octouncuhaptor eugerrei	*Eugerres brasilianus*	Gatun Lake	Mendoza-Franco *et al*., 2008
	Palombitrema heteroancistrium	*Astyanax aeneus*	Nigua River	Mendoza-Franco *et al*., 2009
		Astyanax aeneus	Quebrada Rivers	Mendoza-Franco *et al*., 2009
	Sciadicleithrum panamensis	*Aequidens coeruleopunctatus*	Río Frijolito	Mendoza-Franco *et al*., 2007
	Urocleidoides advenai	*Brachyhypopomus occidentalis*	Aguas Claras River	Mendoza-Franco *et al*., 2009
	Urocleidoides cultellus	*Brachyhypopomus occidentalis*	Aguas Claras River	Mendoza-Franco *et al*., 2009
	Urocleidoides flegomai	*Piabucina panamensis*	Río Frijolito	Mendoza-Franco *et al.,* 2007
	Urocleidoides neotropicalis	*Saccodon dariensis*	Piriati River	Mendoza-Franco & Reina, 2008
	Urocleidoides piriatiu	*Ctenolucius beani*	Piriati River	Mendoza-Franco & Reina, 2008
	Urocleidoides similuncus	*Poecilia gillii*	Río Frijolito	Mendoza-Franco *et al.,* 2007
	Urocleidoides strombicirrus	*Astyanax aeneus*	Quebrada Rivers	Mendoza-Franco *et al.,* 2009
		Astyanax aeneus	Chiriquicito Rivers	Mendoza-Franco *et al.,* 2009

		Astyanax aeneus	Piriati River	Mendoza-Franco *et al*., 2009
	Urocleidoides visiofortatus	*Brachyhypopomus occidentalis*	Aguas Claras River	Mendoza-Franco *et al*., 2009
Acantocephala				
Oligacanthorhynchidae Southwell et Macfie, 1925	*Oncicola* sp.	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
Nematoda				
Anisakidae Railliet & Henry, 1912	*Contracaecum* sp. type 2	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
Cucullanidae Cobbold in 1864	*Cucullanus pimelodellae*	*Pimelodella chagresi*	Río Frijolito	Choudhury *et al*., 2017
Cystidicolidae Skrjabin, 1946	*Spinitectus* sp.	*Brachyrhaphis cascajalensis*	Río Frijolito	Choudhury *et al*., 2017
Dioctophymatidae Railliet, 1915	*Eustrongylides* sp.	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
Heterocheilidae Railliet & Henry, 1915	*Brevimulticaecum* sp.	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
		Oreochromis niloticus	Panama Canal	Roche *et al.,* 2010
Kathlaniidae Lane, 1914	*Falcaustra* sp.	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
		Oreochromis niloticus	Panama Canal	Roche *et al.,* 2010
Oxyuridae Cobbold, 1864	*Cosmoxynemoides* sp.	*Astyanax sp.*	Quebrada Juan Grande	Choudhury *et al*., 2017
Quadrigyridae Van Cleave, 1920	*Quadrigyrus* sp.	*Hoplias microlepis*	Quebrada Juan Grande	Choudhury *et al*., 2017
Quimperiidae Gendre, 1928	*Paraseuratum* sp.	*Hoplias microlepis*	Quebrada Juan Grande	Choudhury *et al*., 2017
Raphidascarididae Hartwich, 1954	*Hysterothylacium* sp.	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
	Raphidascaris sp.	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010
Rhabdochonidae Skrjabin, 1946	*Rhabdochona* sp.	*Bryconamericus emperador*	Río Frijolito	Choudhury *et al*., 2017
Spiruroidea	*Spiroxys* sp.	*Oreochromis niloticus*	Panama Canal	Roche *et al.,* 2010
Copepoda				
Ergasilidae Thorell, 1859	*Ergasilus* sp.	*Oreochromis niloticus*	Panama Canal	Roche *et al.,* 2010
		Vieja maculicauda	Panama Canal	Roche *et al.,* 2010
Pentastomida				
	unidentified	*Vieja maculicauda*	Panama Canal	Roche *et al.,* 2010

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CAPÍTULO 5. Selección mediada por parásitos durante la especiación

Los parásitos tienen efectos sobre la selección natural y sexual que pueden contribuir a la divergencia de sus hospederos. Si distintas poblaciones tienen comunidades de parásitos distintas, y estas diferencias se mantienen en el tiempo, estas pueden promover su divergencia. En este capítulo se presentan los resultados del análisis del objetivo principal del proyecto de doctorado que es estudiar el potencial papel de los parásitos sobre la divergencia del cíclido de Midas. Tomando como base los resultados del segundo capítulo, donde generamos un inventario de las especies de parásitos que infectan a los cíclidos en los lagos de Nicaragua, dimos el siguiente paso, caracterizar los ensambles de parásitos del cíclido de Midas. Encontramos que las poblaciones del cíclido de Midas difieren en sus infecciones parasitarias, incluso en aquellas poblaciones en las que la divergencia entre poblaciones es aun incipiente (Artículo V). Las diferencias en la infección se reflejan en todos los ecomorfotipos paralelos e incluso en los tipos de diferentes coloraciones, evidenciando paralelismos asociados a la historia de vida de cada parásito y disparidad trófica del hospedero. Los ensambles de parásitos están anidados espacialmente, es decir, son más similares entre las poblaciones de un mismo lago, y más similares entre los lagos que mantienen conexión.

5.1 Ensambles de parásitos

Artículo V

Santacruz A, Pérez-Ponce de León G & Barluenga M. Divergent parasite assemblages in young adaptive radiations: parasite-mediated selection in the Midas cichlid? Manuscrito en preparación para *American Naturalist*.

Divergent parasite assemblages in recent adaptive radiations: parasite-mediated selection in the Midas cichlid?

Running title: Parasite assemblages in the Midas cichlid

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Abstract

Parasites exert strong selective pressures on their hosts that can mediate their adaptation to new environments and their evolutionary trajectories. The Neotropical Midas cichlid is an excellent example of rapid and repeated adaptive radiations. This young species complex inhabits several small crater lakes in Nicaragua, displaying evolutionary parallelisms. Here, we explore the potential of parasites to mediate divergence and adaptation in the Midas cichlid. If populations inhabiting different environments are exposed to different parasite communities, and these are temporally stable, this can fuel diversification. We characterized the parasite communities in the Midas cichlids over three consecutive years. We found that abundance and composition of parasite communities are more similar among sympatric than allopatric populations, forming unique assemblages within lakes, stable over time. We uncovered similar abundance patterns among parallel host ecomorphotypes across crater lake radiations. The parallelisms reflect the parasites transmission strategies and life histories of their hosts. Our study sheds light on the hypothesis that parasites have the potential to exert differential and continuous selective pressures in different Nicaraguan settings, and to initiate or enhance diversification among Midas Cichlid populations.

Keywords. speciation, sympatry, phylogeny, infection profiles.

Introduction

Parasites can drive host speciation, by accelerating their speciation rate as a result of higher parasite diversity, or by increasing the differences in the evolutionary trajectories among allopatric populations. Conversely, parasites can limit differentiation and prevent sympatric speciation (Buckling & Rainey, 2002; Betts *et al.*, 2018). Parasite selective pressures have implications for natural and sexual selection of their hosts, e.g., through behavior modulation and habitat selection to avoid or promote parasite infection (Eizaguirre & Lenz, 2010; Mikheev *et al.*, 2013; Demandt *et al.*, 2018; Jolles *et al.*, 2020), or in assortative mating directly or indirectly influencing mating traits (Milinski, 2014). The effect of the parasite is translated into host defense mechanisms as an adaptive response of their host. For example, in vertebrates, highly polymorphic genes of the immune system fluctuate and rapidly change as a related effect to local parasites (Eizaguirre *et al.*, 2012).

Different habitats are associated to different parasite assemblages (MacColl, 2009). The transmission routes for parasite acquisition depend on the life history of the host (Stutz *et al.*, 2014), on traits such as feeding preferences, or social behavior and density of shoals (Hayward *et al.*, 2017). That is, depending on the habitat are the chances of acquiring certain parasites, forming differentiated parasite assemblages, more pronounced in hosts with non-overlapping niches (Meyer *et al.*, 2019). Thus, the contrasting parasite communities represent local adaptations for a host population, which, linked to natural and/or sexual selection, could enhance, maintain or accelerate the differences between populations (Hund *et al.*, 2020), limiting the genetic flow. Parasites could be a major force in the anchoring of mechanisms that allow sympatric speciation. Karvonen & Seehausen (2012) proposed three prerequisites to support the hypothesis of speciation with a parasite-mediated selection: 1) different assemblages of parasites among populations, 2) temporally stable differences and, 3) effects of the parasite on the fitness of its host higher than the effect of other factors.

Cichlid fish exhibit some of the most spectacular adaptive radiations. Hundreds of species have diversified rapidly mainly in the great African lakes (Salzburger *et al.*, 2014), and more recently under sympatric conditions, and on a smaller scale in the crater lakes of Central America (Barluenga & Meyer, 2004, 2010; Barluenga *et al.*, 2006; Kautt *et al.*, 2020). Cichlids show a great propensity to evolve, which might be explained by key evolutionary innovations (Fryer & Iles, 1972), hybridization (Meier *et al.*, 2017; Salzburger, 2018), genomic duplications, standing genetic variation (Brawand *et al.*, 2015) and rapid behavioral changes (Seehausen, 2015). Extrinsic factors have also been proposed to explain cichlids' propensity to radiate, such as ecological opportunity (Seehausen, 2015; Ivory *et al.*, 2016), trophic changes (Burress *et al.*, 2020) and exposure to different parasites (Raeymaekers *et al.*, 2013; Meyer *et al.*, 2019; Gobbin *et al.*, 2020).

The Midas cichlid species complex inhabits the great Nicaraguan lakes and an array of surrounding crater lakes. The crater lakes have been colonized by populations from large lakes, establishing a system analogous to a continent-island-like evolutionary setting (Barluenga & Meyer, 2010; Kautt *et al.*, 2020). The temporal heterogeneity of the crater lake's colonization, and the biotic and abiotic differentiation within lakes, form a geographic mosaic of evolutionary trajectories to study replicated dynamics of the host-parasite interactions. In each of these lakes, the evolutionary young Midas cichlid experienced adaptive radiations through sympatric speciation (Barluenga *et al.*, 2006; Kautt *et al.*, 2020), forming species flocks dwelling differentiated niches (Elmer *et al.*, 2010a). The analogous niches across lakes translate into fish with parallel axes of differentiation; elongated body shapes typical of open water zones, deep bodies that inhabit superficial or deep benthic zones and navigate between rocks (Elmer *et al.*, 2014; Kusche *et al.*, 2014; Kautt *et al.*, 2016), thick lips linked to a diet between the rocks (Machado-Schiaffino *et al.*, 2014), or pharyngeal jaw shapes that vary depending on soft, hard or mixed diets (Muschick *et al.*, 2011).

Cichlids harbor a great diversity of parasites in the freshwater systems of Nicaragua (Santacruz *et al.*, 2022b). Recent extensive work characterized all the macroparasites in the cichlids of the great lakes and crater lakes of Nicaragua (Santacruz *et al.*, 2022a) and described a few new species (Santacruz *et al.*, 2020, 2021). In accordance with a continent-island like model, the great lakes harbor a more diverse parasite community than the crater lakes. The parasite diversity in some crater lakes is more differentiated than the others or harbor unique parasite species. The Midas cichlid is infected by the typical parasites of Middle American cichlids. However, across the lakes harbors a core parasite fauna (*sensu* Pérez-Ponce de León & Choudhury, 2005), which comprises distant phylogenetically related parasites, with different life cycles and transmission strategies (Santacruz *et al.*, 2022a).

Here, we investigate the potential contribution of parasite assemblages to the repeated and parallel divergence of the Midas cichlid in several crater lakes. To this end we analyzed the parasite assemblages in the Midas cichlid species complex across the Nicaraguan lakes and investigated the patterns of variation according to parasite richness, abundance, and phylogenetic diversity. We examined temporal, geographic, and biotic features influencing parasite assemblages, as well as parallelism in diversity and assemblage patterns.

Methods

Study system

The Midas cichlid (*Amphilophus* spp.) is a polymorphic species complex with 13 described species, although more remain to be characterized. These fish are distributed in freshwaters of Nicaragua and North Costa Rica, being most abundant in the great Nicaraguan lakes and several lakes of volcanic origin (Fig. 1a). The fauna in the crater lakes originated recently (between 800 to 4,700 years ago; Kautt *et al.*, 2020). Fish radiated within the lakes following common axes of ecological differentiation (Fig. 1b) along the benthic-limnetic gradient with varying body shapes, along alternative prey types with varying pharyngeal jaw shape and lip size, and also with alternative background coloration, dark or gold (Muschick *et al.*, 2011; Elmer *et al.*, 2014; Kusche *et al.*, 2014; Kautt *et al.*, 2016; Machado-Schiaffino *et al.*, 2017). Benthic and limnetic species are found in the relatively deep crater lakes Xiloá and Apoyo (Elmer *et al.*, 2014). Fish with molariform and papilliform teeth in the pharyngeal jaws are found in all lakes except crater Lake Masaya. Fish with thick lips have been described in the great lakes, where they are recognized as a separate species (Barlow & Munsey, 1976), and similar forms are found in several crater lakes (Apoyeque, Masaya, Xiloá) where they are still considered a phenotype within the species *A. citrinellus* (Manousaki *et al.*, 2013). Polychromatic fishes are found in all lakes (but crater Lake Apoyo) and within several ecomorphotypes (Barlow, 1976). Patterns of repeated evolution with convergent forms are found in several lake radiations (Elmer *et al.*, 2014). We focused on the large lakes and five crater lakes and on a total of eight Midas cichlid described species.

We have recently characterized the Nicaraguan lakes macroparasite fauna (Santacruz *et al.*, 2022a). In the Midas cichlid we have identified 22 parasite taxa, with a set of common species across lakes representing the core parasite fauna composed by eight parasite taxa (Fig. 1c). Common parasites are mainly helminths (Trematoda, Monogenea, Acanthocephala and Nematoda), and also copepods, organisms with contrasting life histories, transmission strategies and levels of host specificity.

Sampling

Samples were collected from the great Nicaraguan lakes Managua and Nicaragua, and five crater lakes, Asososca León, Apoyeque, Xiloá, Masaya and Apoyo. Fish were collected over three consecutive years (2017 - 2019), at the end of the rainy season (November-December). In some locations, only data from one (crater Lake Apoyeque) or two field seasons (crater Lake Masaya) is available. Sampling was performed using gill nets, and with the help of local fishermen. Collection permits (No. 001-012015) were provided by the Ministry of Natural Resources (MARENA) of Nicaragua. Fish were euthanised following ethical procedures, with an overdose of tricaine methanesulfonate (MS-222) on cold water. Each fish was photographed in a standardized lateral position for species identification, weighed and sexed. Fin clips were preserved in 100% ethanol for DNA analysis. Ecto- and endoparasites were screened in the field immediately after euthanization with a Leica EZ4 stereomicroscope. First, the fish external surface (skin, eyes, and mouth) was assessed. Then, fish were dissected, and internal organs (gut, mesentery, muscle and gall, swim, and urinary bladders) were analyzed. All recovered parasites were rinsed in saline solution and stored in 100% ethanol for DNA extraction. Representative specimens of each parasite taxa were fixed in nearly boiling 4% formalin for morphological analysis. Gill arches were dissected and stored in 100% ethanol. Later, in the laboratory, the gills were screened for ectoparasites using a Leica EZ4 stereomicroscope.

Parasite diversity

We calculated the diversity of parasites within individual hosts, i.e., the infracommunity (Bush *et al.*, 1997) of the host ecomorphotypes, i.e. limnetic, benthic or rocky within each lake. For each infracommunity we calculated *richness*, as the number of parasite taxa, *abundance*, as the number of individuals of each parasite taxa, and *infection intensity*, as the total number of a given parasite in infected hosts (Rózsa *et al.*, 2000). We also calculated the phylogenetic diversity of the parasite communities. We calculated the phylogenetic species evenness (PSE) index (Helmus *et al.*, 2007) to consider phylogenetic relatedness and species evenness (richness and relative species abundance) for each infracommunity. This index reaches its maximum value (i.e., 1) if all parasite species are equally abundant, and their phylogeny has a star topology. The model assumes that branch lengths are proportional to the evolutionary divergence among species.

We first reconstructed the phylogenetic relationships among the parasite taxa infecting the Midas cichlid. We generated a matrix using a region of a common genetic marker available for all the parasites, the nuclear 28S. The sequences were recovered from the GenBank using the species or congeneric species (Table S1). Sequences were aligned with the Clustal Omega platform (https://www.ebi.ac.uk/Tools/msa/clustalo/; McWilliam *et al.*, 2013). The best fit model of molecular evolution for each dataset was calculated with ModelFinder (Kalyaanamoorthy *et al.*, 2017) using the Bayesian information criterion (BIC). Phylogenetic reconstruction was performed with Maximum likelihood (ML) in IQ-TREE v.1.6.2 (http://iqtree.cibiv.univie.ac.at/), using Ultrafast bootstrap with 10,000 iterations and SH-aLTR branch test with 10,000 replicates (Nguyen *et al.*, 2015; Hoang *et al.*, 2017). Then, using as input the phylogeny and the abundance of each parasite taxa, we calculated the PSE for the infracommunity using the *pse* function implemented in the R package ‘picante’ (Kembel *et al.*, 2010).

Statistical analysis

All the statistical analyses were run in R version 4.1.0 (R Core Team, 2018). We compared richness and PSE index across host populations using linear models with host species as fixed effect, and additional post hoc tests for pairwise comparisons between host populations.

To analyze spatial and temporal variation in the parasite assemblages across lakes and populations we first built a Bray-Curtis similarity matrix based on abundance data, the matrix was calculated for all parasites, and for endoparasites separately. Then, we calculated differences among lakes, host populations and sampling years with a permutational multivariate analysis of variance (PERMANOVA), using the matrix for all parasites. The matrices were used for an ordination analysis using non-metrical multidimensional scaling (NMDS). All graphical outputs were generated using ‘ggplot2’ package (Wickham, 2009). 95% confident ellipses (Fox & Weisberg, 2011) were calculated with the *stat_ellipse* function of the same package.

We assessed parallelisms according to ecological features of the fish populations. We thus classified each fish according to body shape (benthic/limnetic), pharyngeal teeth type (molariform/papilliform), lips size (thin/thick) and body color (dark/gold). Then**,** to identify the host and abiotic features explaining among-lake and among-population that predict the parasite load of a given parasite taxon, we used negative binomial mixed models GLMM (Generalized Linear Mixed Models). The abundance of each member of the core parasite fauna was used as response variable using a negative binomial probability distribution as a function of host population nested with lake. Sampling year (nested with site), sex, color, body shape, pharyngeal teeth type and lips size were included in the model as fixed effects and standard length as a covariate.

Results

We collected 509 Midas cichlid individuals from 16 populations in seven lakes. We isolated more than 60,000 individual parasites, and identified 22 parasite taxa, mainly helminths. We recovered the core parasite fauna of the Midas cichlid formed by eight taxa: the trematodes *Crassicutis cichlasomae, Oligogonotylus manteri, Saccocoelioides* spp., the monogenean *Sciadicleithrum mexicanum*, the acanthocephalan *Neoechinorrynchus costarricense*, the larval nematodes *Contracaecum* spp., the adult nematode *Procamallanus barlowi* and the copepods of the family Ergasilidae (see the complete list of parasites in Table S2).

The mean parasite richness per individual host was 3.5 ± 1.35, ranged between 0 and 8, with the lowest value of 1 ± 0 in the thick-lipped *A. citrinellus* from crater Lake Masaya, and the highest value 4.7 ± 1.36 in the limnetic *A. zaliosus* from crater Lake Apoyo (Fig. 2a). The mean parasite diversity assessed with the PSE index was 0.22 ± 0.16, ranged between 0 and 0.61 (Fig. 2b). The highest mean PSE value (0.37) was found in both the benthic *A. citrinellus* from crater lake Asososca León and the thick-lipped *A. citrinellus* from crater Lake Apoyeque. The lowest mean PSE value (0.06) was found in the benthic *A. astorquii* from crater Lake Apoyo. PSE varied among lakes (F= 12.17, p < 0.0001) and host populations (F= 7.519, p < 0.0001). The abundance and intensity of the core parasite fauna was heterogeneous across lakes (Table 1, Fig. 3). Some parasite taxa (e.g., the trematodes *C. cichlasomae* and *O. manteri*) were fairly similar across populations, while others showed important differences (e.g., larval nematodes or monogeneans).

Spatial and temporal variation of parasite communities

The ordination analysis showed nested parasite assemblages, with a signature to the lake of origin (Fig. 4a), i.e., parasite communities are more similar among sympatric than among allopatric host populations. The communities are less differentiated among lakes if we omit the ectoparasite values. When parasite communities are partitioned according to host ecomorphotype, i.e. limnetic, benthic or rocky, the infracommunities are more differentiated (Fig. 4b–e). We found no differences in the annual replicates (PERMANOVA, F = 0.716, p = 0.602). Differences are found among lakes (PERMANOVA, F = 7.169, p < 0.001**)**, and host populations (PERMANOVA, F = 3.064, p < 0.001).

Infection patterns in parallel host ecomorphotypes

Differences in the infection profiles were found between host populations when each parasite taxon was considered separately, except in the acanthocephalan *N. costarricense* (Table 2). For this species, differences were best explained by the effect of the lake. The lake of origin showed an effect for *C. cichlasomae*, *O. manteri* and the copepods. Abundance variation in *P. barlowi* was significantly associated to host standard length. None of the abundances of each core parasite taxa showed differences explained by year.

We first conducted an overall analysis of the abundance of individual parasite taxa according to the axes of morphological differentiation explored among the lakes, which showed that benthic and limnetic body shapes were significantly different in four parasite taxa: larval nematodes, *Saccocoelioides* spp., *O. manteri,* and *S. mexicanum* (Fig. S1a). In the thin-lipped and thick-lipped phenotypes, the abundances of three parasite taxa (*Saccocoelioides* spp., *O. manteri*, *O. manteri* and *N. costarricense*) were significantly different (Fig. S1b). In the pharyngeal jaw phenotype comparison, we found significant differences in four parasite taxa: *Saccocoelioides* spp., *C. cichlasomae*, *O. manteri*, and larval nematodes (Fig. S1c). In the gold and dark phenotypes, the abundances of three parasite taxa were different. *Sciadicleithrum mexicanum* and *Saccocoelioides* spp. were significantly less parasitized in the gold than in the dark phenotype, but the copepods had higher parasite loads in the gold phenotype (Fig. S1d).

We conducted analyses to explore the role of parasites in specific cases of parallel evolution of the Midas cichlid, by considering the axes of morphological differentiation. For instance, in the deep crater lakes Xiloá and Apoyo we analyzed the parallel benthic and limnetic ecomorphotypes. We found parallelisms when considering individual infections by a given parasite taxon (Fig. 5). Significative differences are exhibited by nematode larvae which display the highest infection in the limnetic populations in both lakes, with abundances as high as 1800 parasites in a single individual host, in contrast to relatively less infected benthic/rocky forms (Fig. 5a). The ecomorphotypes with thin and thick-lipped phenotypes were analyzed in the great lakes and crater Lake Apoyeque. The thin-lipped *A. citrinellus* versus the thick-lipped *A. labiatus* from the great lakes displayed parallel abundances of the trematode *O. manteri* (Fig. 5b). Instead, the thin-thick lipped phenotypes in crater lake Apoyeque did not differ in abundances in any of the parasite taxa, but *O. manteri* incipiently changes the infection pattern with respect to the great lakes, with minor abundance in the thick-lipped phenotype. When considering the pharyngeal jaw phenotype in populations of the crater Lake Asososca León, the infection with *C. cichlasomae* was significantly different, with higher infection levels in the *A. citrinellus* molariform (Fig. 5c). The comparison also made in the crater Lake Asososca León between Midas cichlids with dark and gold colorations showed significantly higher infection levels for *C. cichlasomae* in the dark *A. citrinellus* (Fig. 5d).

Discussion

In this study we analyzed the contribution of different parasite assemblages to repeated and parallel divergence of the Midas cichlid in several crater lakes of Nicaragua. We found support for assumptions under which parasites would be likely to contribute to host divergence by comparing host populations with divergent ecomorphotypes: 1) different host populations are infected by different parasite assemblages, even occurring in sympatry, 2) the parasite loads are related to the host trophic niche, and 3) the infection pattern is maintained over time. Thus, the contrasting parasite assemblages may contribute to trigger and maintain the isolation of sympatric host populations since incipient speciation stages in this study model.

The Midas cichlid populations harbor a core parasite fauna, represented by parasites occurring in almost all the host populations (Santacruz *et al.*, 2022a). However, the combination of parasite composition and parasite loads differed among host populations, giving specific signatures and building unique parasite assemblages, which are lake-specific, and habitat-specific within lakes. The observed pattern suggests that may after a single event of colonization, the host along with its parasites were shaped by stochastic or deterministic factors in a similar way in each crater lake. Besides the asynchronous time of colonization of the crater lakes, they differ in the availability of niches and environmental variables (Barlow, 1976; Stauffer *et al.*, 2008; Torres-Dowdall *et al.*, 2017). The similarity of the parasite communities in each lake is in accordance with the spatial clustering of the Midas cichlid species complex defined by Kautt *et al.* (2020). The parasite communities of the great lakes Managua and Nicaragua maintain a more diffuse differentiation, overlapping the spatial variation of the crater Lake Masaya. The remaining crater lakes display their own identity, being Asososca León, Apoyeque and Apoyo the more differentiated. Spatial nested parasite communities are widely documented in other study models (González & Poulin, 2005; Moss *et al.*, 2019). This means that parasite assemblages are built with a subset of all the parasite diversity that infects a host species (Poulin & Valtonen, 2001). Therefore, heterogeneous parasite communities across the distribution range of a host species represent a common pattern (Poulin & Morand, 1999; Poulin & Valtonen, 2002), given the scale-dependent local variations of the host traits or environmental conditions (Bolnick *et al.*, 2020).

The Midas cichlid exhibit parasite signatures in ecological axes of differentiation such as body shape, pharyngeal jaw and lip size (Muschick *et al.*, 2011; Elmer *et al.*, 2014; Kusche *et al.*, 2014; Machado-Schiaffino *et al.*, 2014; Kautt *et al.*, 2016). In the crater lakes where the clearest parallelisms have been evidenced, Apoyo and Xiloá (Elmer *et al.*, 2014), the body shape associated with deep or superficial benthic environments, and limnetic forms dwelling open waters, show a similar infection pattern. The limnetic forms have higher infection intensities in larval nematodes, in accordance with a more predatory diet. But higher intensities of trophic parasites could also indicate fish populations more predated by the parasite definitive host (Johansen *et al.*, 2019). The infection pattern changes when considering parasites with other life histories and transmission strategies. The shallow benthic populations in both lakes show highest infection intensities caused by monogeneans; these parasites have direct life cycles, with no intermediate hosts. The transmission strategy of monogeneans is contact-dependent, in accordance with the more cohesive and dense populations of the shallow benthic fishes. The parallelisms are also replicated in thick lips populations of Midas cichlid in both great lakes which have similarities in the infection patterns. For instance, the trematode *O. manteri* (which uses a snail as intermediate host, see Scholz *et al.*, 1994), exhibits higher parasite loads in thick-lipped fish in both lakes, in contrast to the thin-lipped *A. citrinellus*. The opposite pattern of infection with the same parasite is exhibited in thick-lipped fish from crater Lake Apoyeque.

Crater Lake Apoyeque provides an optimal scenario to test whether parasites facilitate divergence in populations in the midst of the speciation process, because it is a very recent lake colonization (Kautt *et al.*, 2020). This crater lake harbors two non-genetically differentiated ecomorphotypes (Elmer *et al.*, 2010b; Manousaki *et al.*, 2013). The parasite communities are differentiated from the other lakes, but ecomorphotypes show only incipient differences in infection patterns, however, specimens were studied only in one sampling season. Even so, parasites can be a factor that contributes to other drivers of speciation and complements the process.

The Midas cichlid is polychromatic, with the gold coloration as the most conspicuous trait, giving rise to extraordinary and rare phenotypes. This character occurs in any of the ecomorphotypes, benthic or limnetic forms, thick or thin lips. We found that gold colorations tend to be infected with lower parasite loads, independent of the ecomorphotype or the lake population. The parallelisms in the parasite assemblages associated to the color of the host may be linked to a similar habitat, diet and behaviors in the Midas gold populations. The Midas cichlids hatch with a dark coloration (Barlow, 1976; Sowersby *et al.*, 2015). Interestingly, the transition from dark to gold can last even years maintaining dark regions or begin years later in the fish life (Henning *et al.*, 2013; Lehtonen *et al.*, 2015), implying that gold fish could reproduce with their dark coloration. Some studies have reported assortative mating in the coloration and genetic differentiation between dark and gold forms (Elmer *et al.*, 2009). Gold forms appear in low frequencies in all lakes, being more common in turbid waters, and absent in the clear waters of crater Lake Apoyo (Barlow, 1983). In other fish species, coloration is associated to the transmission strategy of their parasites; a more colorful fish is usually more intensively predated, representing a more successful transmission for a parasite that matures in the predatory species represented by the definitive host (Johansen *et al.*, 2019). In other fish species, coloration has been associated with the physical condition of the animal, and with an "honest" signal correlated with immune protection against parasites (Johansen *et al.*, 2019). Therefore, color is considered a phenotypic ornamentation linked to mate choice. For instance, the presence of a parasite could cause changes in the fish coloration, which can be perceived by the female (Milinski & Bakker, 1990).

In this study we report higher rates of infection than those reported in African cichlids. For instance, in studies on parasites of Lake Victoria cichlids, the highest intensity reported was 152 nematodes in a single *Pundamilia pundamillia* or 59 *Cichlidogyrus* monogeneans in one host of *Astatoreochromis alluaudi* (Gobbin *et al.*, 2020), which contrast with the 1800 larval nematodes found in a single specimen of the Nicaraguan *A. sagittae* from crater Lake Xiloá, or the 762 *S. mexicanum* monogeneans and more than 1000 *Saccocoelioides* spp. trematodes infecting single individuals of *A. astorquii* from crater Lake Apoyo. The infection pattern might rely on more tolerant or susceptible hosts, or from the parasite perspective, more successful infections.

The extraordinary diversification of cichlid fish has been linked to parasite-mediated selection in some studies (Raeymaekers *et al.*, 2013; Karvonen *et al.*, 2018; Gobbin *et al.*, 2020). Contrasting parasite communities in different habitats generate local adaptations; for instance, an immune system able to cope with the current parasites (Eizaguirre *et al.*, 2012). The Midas cichlid possess extraordinary diversity in the major histocompatibility complex genes (MHC) (Hoffman *et al.*, 2017), which are lake-specific and habitat-specific (Bracamonte *et al.*, 2022). MHC allelic diversity has been proposed to match the diversity of parasite communities of a given host population (Meyer *et al.*, 2019). In conjunction, a host population facing different parasite communities may impose changes in their immune system, acting as a magic trait mediating host divergence. The next step in our studies, is to search for a match between the parasites and the MHC of the Midas cichlid.

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Author contributions.

Competing interests

The authors declare no competing interests.

Figure legends

Figure 1. **a**, Sites of the Midas cichlid populations sampled along the great lakes (L) Nicaragua and Managua and fiver crater lakes (Cl). **b**, Axes of morphological differentiation. **c**, The core parasite fauna of the Midas cichlid.

Figure 2. Relative abundance of the parasite core fauna in the Midas cichlid across the Nicaraguan lakes.

Figure 3. Richness and PSE values in the Midas cichlid populations.

Figure 4. NMDS of endo- and ectoparasite communities in the Midas cichlid, using Bray-Curtis dissimilarities. Each triangle represents the community of parasites of a species of the Midas cichlid in its respective coloration: dark or gold. The proximity between the samples reflects the similarity in the parasite communities. The polygons indicate the parasite communities within lakes.

Figure 5. Parallelisms in the infection profiles. **a**, Body shape (benthic/limnetic) in the crater lakes Xiloá and Apoyo infected by larval nematodes, **b**, lips shape (thin/thick) across the Great lakes and crater lake Apoyeque infected by the trematode *O. manteri*, **c**, pharyngeal jaw (molariform/papilliform) and **d**, color (dark/gold) in the populations from crater Lake Asososca León infected with the trematode *C. cichlasomae*.

Table captions

Table 1. Mean abundance (A) with range and mean intensity (I) of the core parasite fauna in the Midas cichlid.

Table 2. Generalized linear models for infection levels (abundance) of th core parasite

fauna in 16 Midas cichlid populations.

Supplementary material

Figure S1. Parallelisms in the infection profiles. **a**, Body shape (benthic/limnetic), **b**, lips shape (thin/thick), **c**, pharyngeal jaw (molariform/papilliform) and **d**, color (dark/gold).

Table S1. GenBank accession number of the 28S region of the taxa used to reconstruct the phylogeny

Table S2. List of parasites infecting the Midas cichlid across the great lakes and crater lakes of Nicaragua.

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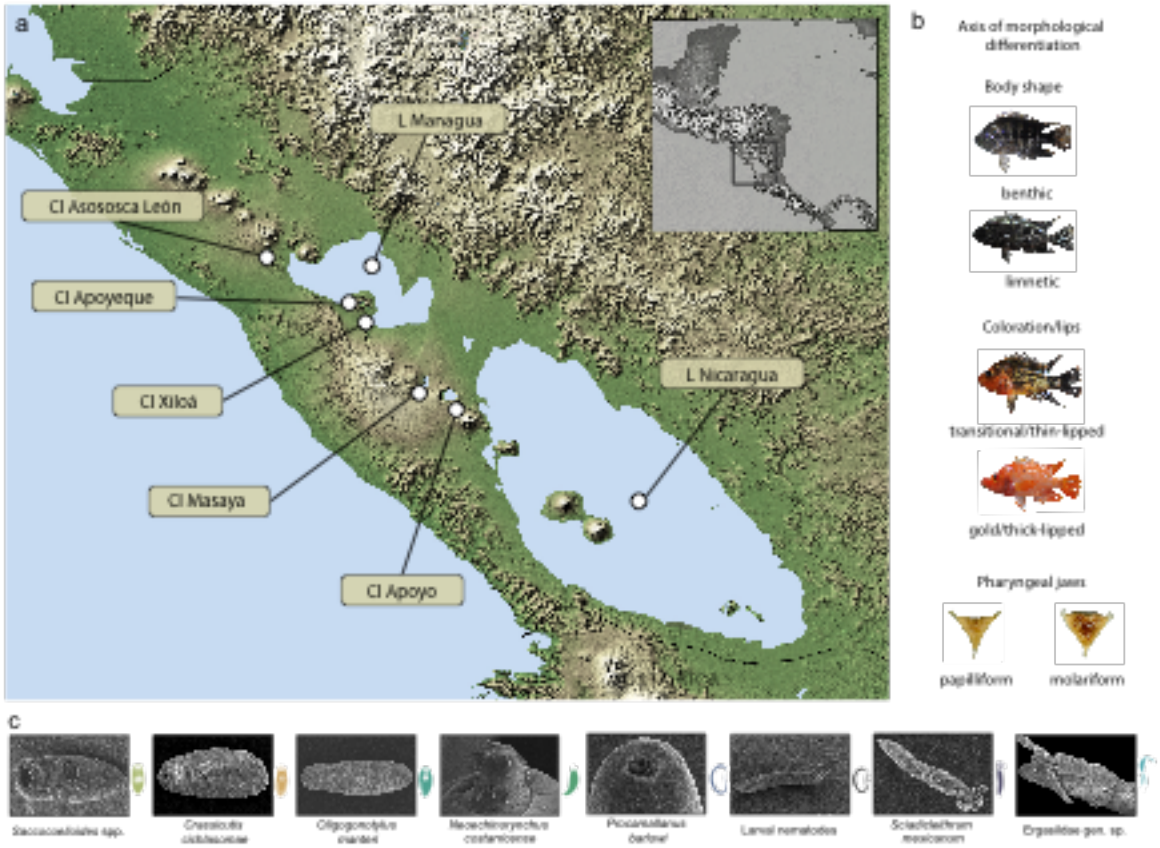

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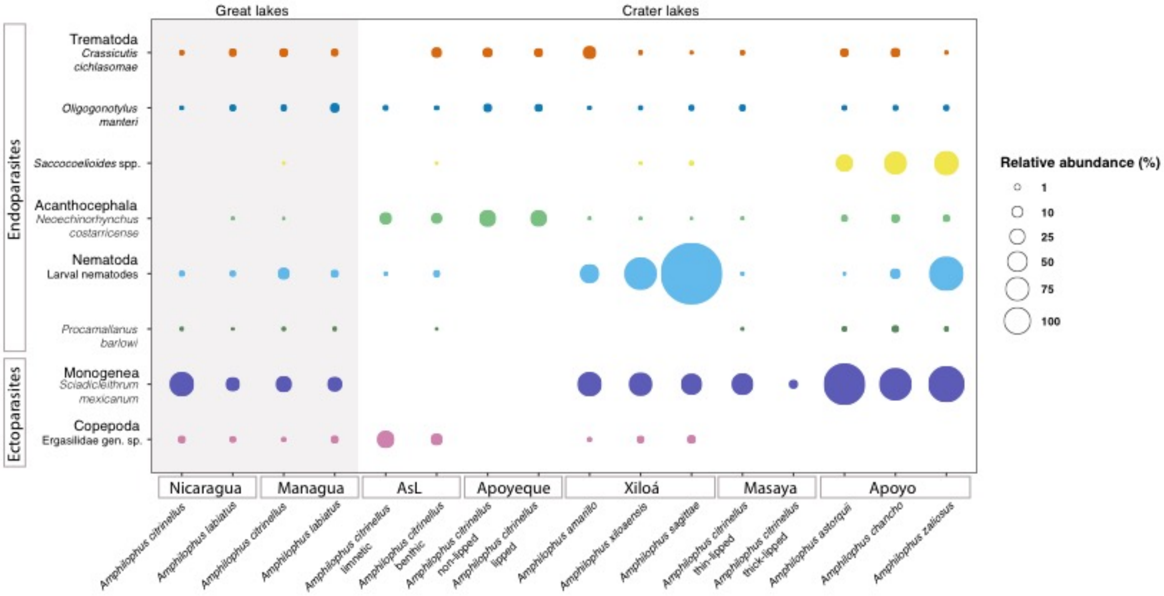

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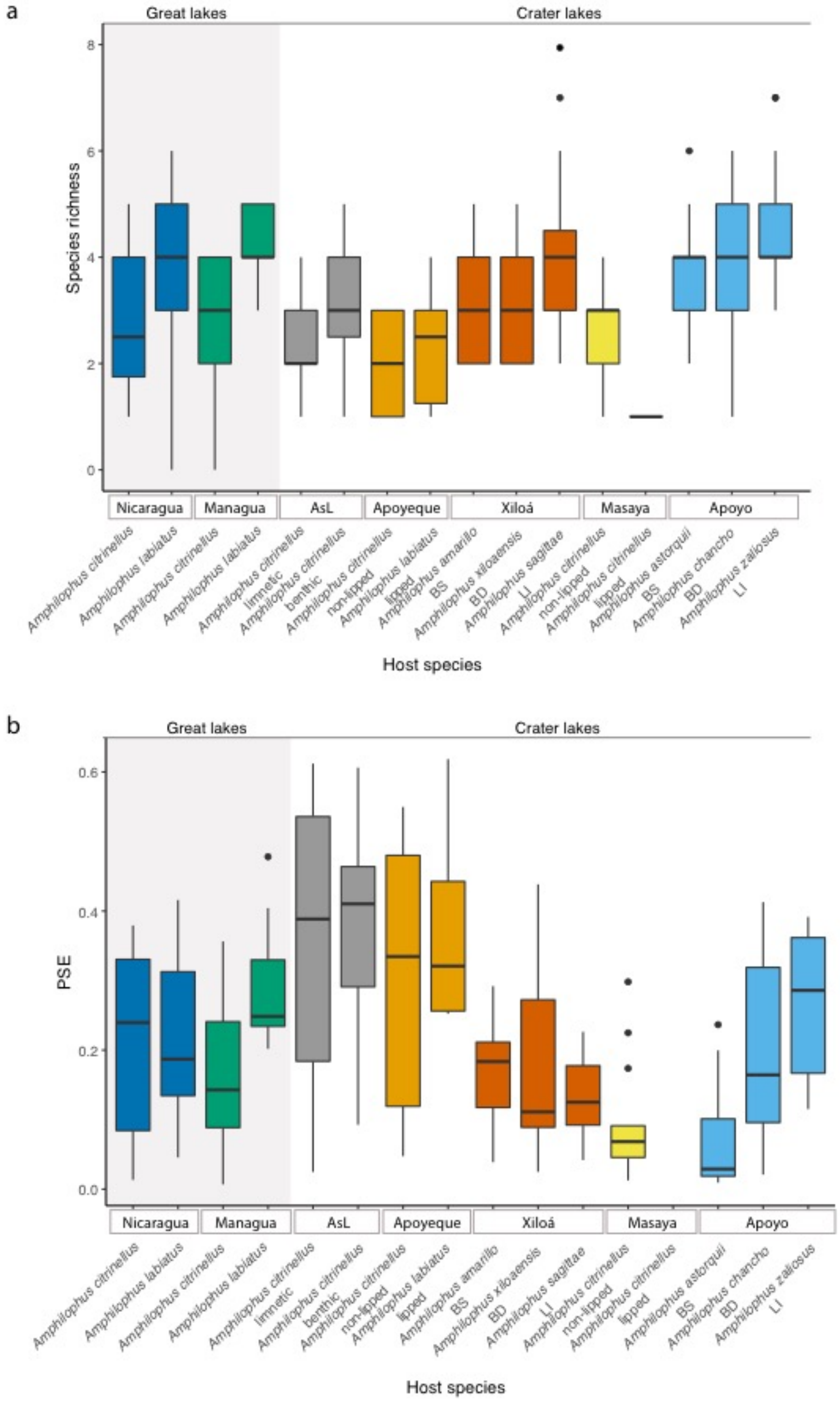

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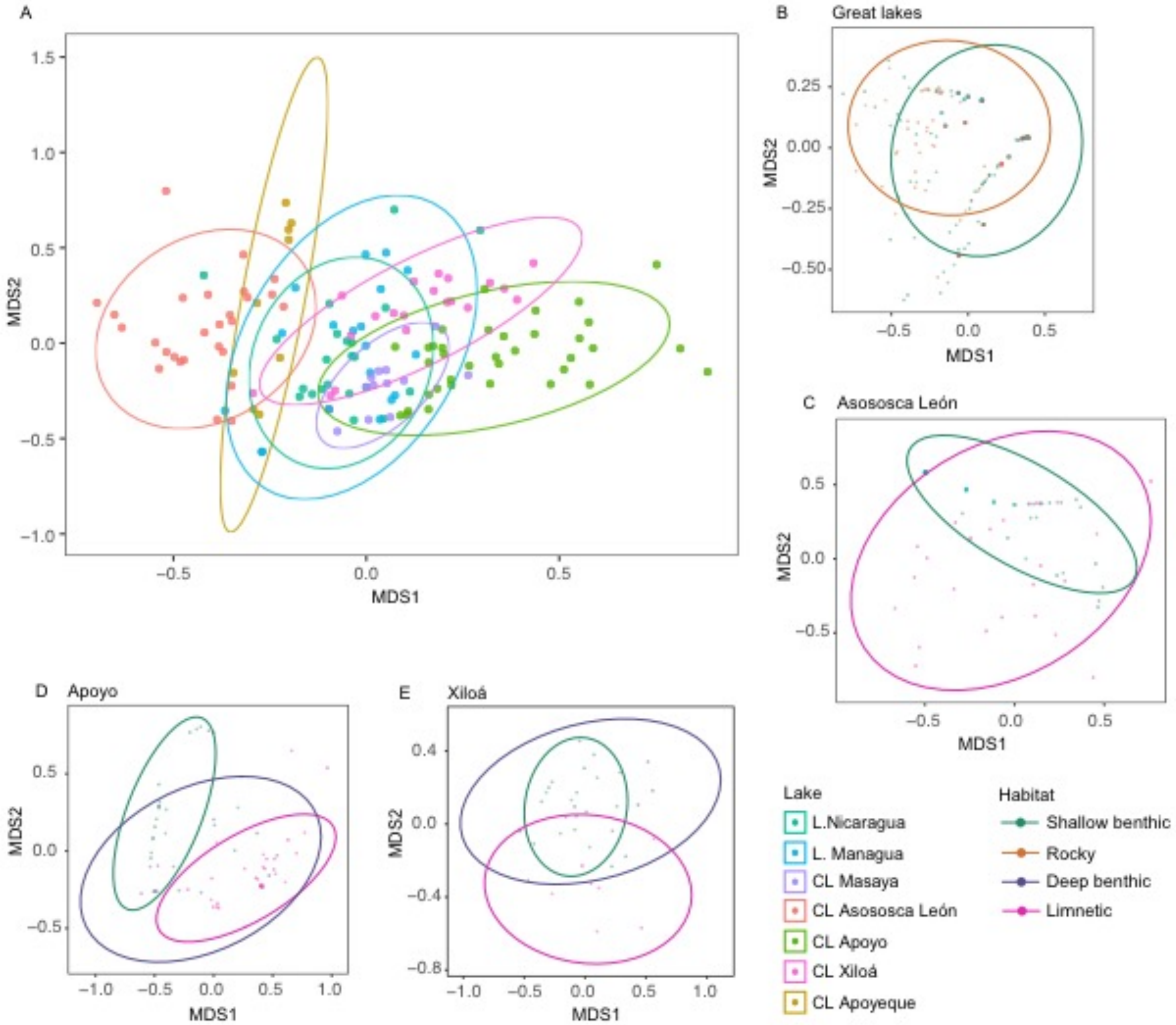

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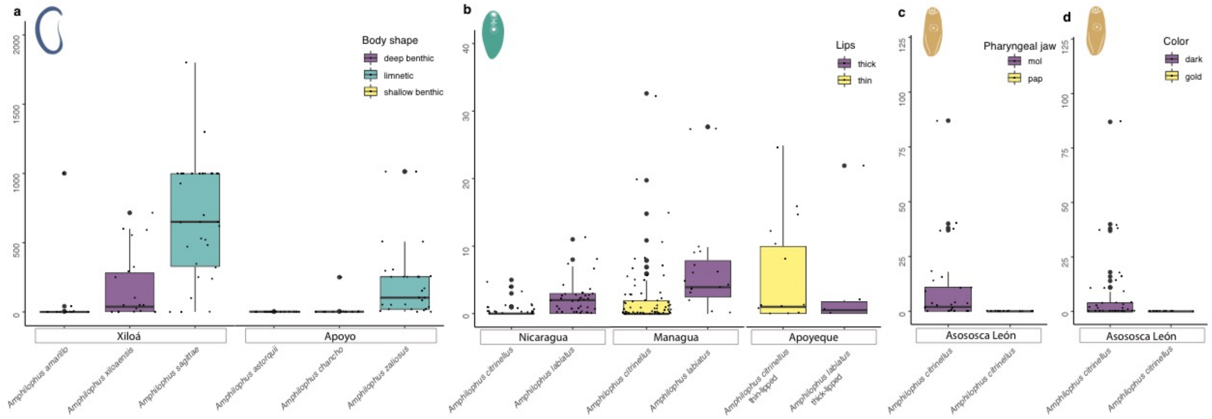

Table 1. Mean abundance (A) with range and mean intensity (I) of the core parasite fauna in the Midas cichlid. BS;

	Trematoda						Acanthocephala		Nematoda	
	Crassicutis cichlasomae		*Oligogonotylus manteri*		*Saccocoelioides* spp.		*Neoechynorhynchus costarricense*		Larval nematodes	
Host population	A	I	A	I	A	I	A	I	A	I
Nicaragua										
Amphilophus citrinellus	0.8 (0−10)	3.4	0.4 (0−5)	1.9	0	0	0	0	1.4 (0−30)	5
Amphilophus labiatus	4.2 (0−43)	7	2.0 (0−11)	2.9	0	0	0.1 (0−1)	1	1.7 (0−43)	10.5
Managua										
Amphilophus citrinellus	5.6 (0−125)	11.78	2.1 (0−33)	4.7	0.05 (0−3)	2.5	0.04 (1)	1	14.0 (0−1101)	48.36
Amphilophus labiatus	3.7 (0−18)	10.1	6.7 (0−28)	8.6	0	0	0	0	4.3 (0−13)	4.7
Asososca León										
Amphilophus citrinellus limnetic	0	0	1.2 (0−11)	5.1	0	0	15.8 (0−62)	18.1	0.3 (0−2)	1.4
Amphilophus citrinellus benthic	9.5 (0−87)	16.1	0.5 (0−4)	2	0.05 (0−2)	2	11.1 (0−62)	15.1	2.7 (0−33)	9.3
Apoyeque										
Amphilophus citrinellus thin-lipped	8.0 (0−32)	11.1	5.2 (0−25)	9	0	0	36.3 (0−100)	46.7	0	0
Amphilophus citrinellus thick-lipped	5.8 (0−16)	7	4.1 (0−22)	8.3	0	0		34.3 (0−200)	41.2	0
Xiloá										
Amphilophus amarillo (BS)	18.4 (0−65)	19.3	0.3 (0−3)	2.3	0	0	0.1 (0−1)	1	47.8 (0−1001)	175.5
Amphilophus xiloaensis (BD)	0.52 (0−5)	2.2	0.7 (0−5)	2.2	0.09 (0−2)	2	0.09 (0−1)	1	164.6 (0−716)	213.1
Amphilophus sagittae (LI)	0.1 (0−2)	1.2	1.4 (0−5)	2.3	0.4 (0−7)	3.4	0.05 (0−1)	1	663.7 (0−1801)	752.2
Masaya										
Amphilophus citrinellus thin-lipped	0.7 (0−10)	3.5	2.2 (0−12)	3.15	0	0	0.19 (0−4)	3	0.2 (0−6)	3.5
Apoyo										
Amphilophus astorquii (BS)	5.2 (0−22)	6	1.0 (0−4)	2.2	36.2 (0−750)	78	2.9 (0−40)	10.3	0.07 (0−1)	1
Amphilophus chancho (BD)	7.3 (0−76)	10.5	1.2 (0−13)	4.1	72.8 (0−1000)	105.2	5.9 (0−30)	10.3	9.6 (0−246)	83.6
Amphilophus zaliosus (LI)	0.2 (0−2)	1.1	1.5 (0−9)	3.8	85.9 (0−1000)	109.1	3.4 (0−62)	10.4	187.8	229.5

Nematoda		Monogena		Copepoda	
Procamallanus barlowi		*Sciadicleithrum mexicanum*		Ergasilidae gen. sp.	
A	I	A	I	A	I
0.2 (0-5)	2.4	86 (7−244)	86	3.62 (0−8)	7.25
0.02 (0−1)	1	22.4 (6−41)	22.4	2.1 (0−8)	3.2
0.2 (0−6)	2.2	31.3 (0−119)	49.66	0.6 (0−4)	2.6
0.3 (0−5)	2.3	25 (0−73)	33.3	4 (0−10)	4.5
0	0	0	0	38.1 (0−122)	39.9
0.02 (0−1)	1	0	0	14.1 (0−52)	14.9
0	0	0	0	0	0
0	0	0	0	0	0
0	0	84.8 (26−156)	84.8	0.5 (0−2)	1.6
0	0	78 (8−168)	78	3.5 (0−12)	6.5
0	0	60.9 (10−142)	60.9	5.2 (0−44)	9.4
0.1 (0−4)	4	64.9 (24−126)	64.9	0	0
0.8 (0−8)	3.2	284.4 (40−762)	284.4	0	0
2.9 (0−41)	7.7	164.1 (22−518)	164.1	0	0
0.6 (0−6)	2.2	209 (16−554)	209	0	0

Table 2. Generalized linear models for infection levels (abundance) of th core parasite fauna in 16 Midas cichlid populations.

					SQRT	
Parasite	Effect	df	F	p-value	F	p-value
Crassicutis cichlasomae	Host species	8	4.9135	**9.64E-06**	9.7503	**4.01E-12**
	Lake	4	2.4728	**0.04446**	4.4111	**0.001745**
	Year	1	0.4999	0.48006	0.0266	0.870445
	SL	1	0.5818	0.44615	0.2258	0.63496
	Color	2	0.0602	0.94161	0.4996	0.607272
Oligogonotylus manteri	Host species	8	2.3193	**0.0197454**	3.1068	**0.002155**
	Lake	4	5.591	**0.0002316**	3.7126	**0.005703**
	Year	1	0.3607	0.5485362	2.1681	0.141881
	SL	1	0.7866	0.3757901	0.5834	0.445561
	Color	2	1.6584	0.1920757	1.0915	0.336968
Saccocoleioides spp.	Host species	8	7.5934	**2.66E-09**	21.8569	**<2e-16**
	Lake	4	0	1	0.0026	1
	Year	1	1.0516	0.3059	0.0576	0.8104
	SL	1	1.4535	0.2289	0.4388	0.5082
	Color	2	0.0191	0.9811	0.0049	0.9951
Neoechynorrynchus costarricense	Host species	8	1.4572	0.1719	4.7747	**1.47E-05**
	Lake	4	35.063	**<2e-16**	51.0746	**2.20E-16**
	Year	1	1.0175	0.3138	2.0393	0.1543
	SL	1	0.3878	0.5339	0.8101	0.3688
	Color	2	0.0269	0.9735	0.106	0.8994
Procamallanus barlowi	Host species	8	4.5348	**3.05E-05**	7.5065	**3.46E-09**
	Lake	4	0.0402	0.99692	0.3379	0.852315
	Year	1	3.7019	0.05524	5.8325	**0.016293**
	SL	1	4.3034	**0.03884**	6.8003	**0.009541**
	Color	2	0.1046	0.90069	0.2184	0.80392
Sciadicleithrum mexicanum	Host species	7	24.988	**<2e-16**	47.0737	**2.20E-16**
	Lake	4	1.6741	0.1596	16.8406	**3.09E-11**
	Year	1	0.1277	0.7213	0.2581	0.6122
	SL	1	0.0012	0.9724	0.0254	0.8736
	Color	2	0.0622	0.9397	0.3858	0.6806
Copepodos	Host species	7	3.0001	**0.005865**	8.2688	**2.29E-08**
	Lake	4	14.062	**1.32E-09**	36.0758	**< 2.2e-16**
	Year	1	0.1959	0.658802	1.4585	0.2293
	SL	1	0.0384	0.844859	0.0907	0.7638

	Color	2	0.0364	0.964244	0.3834	0.6823

Figure S1. Parallelisms in the infection profiles. **a**, Body shape (benthic/limnetic), **b**, lips shape (thin/thick), **c**, pharyngeal jaw (molariform/papilliform) and **d**, color (dark/gold).

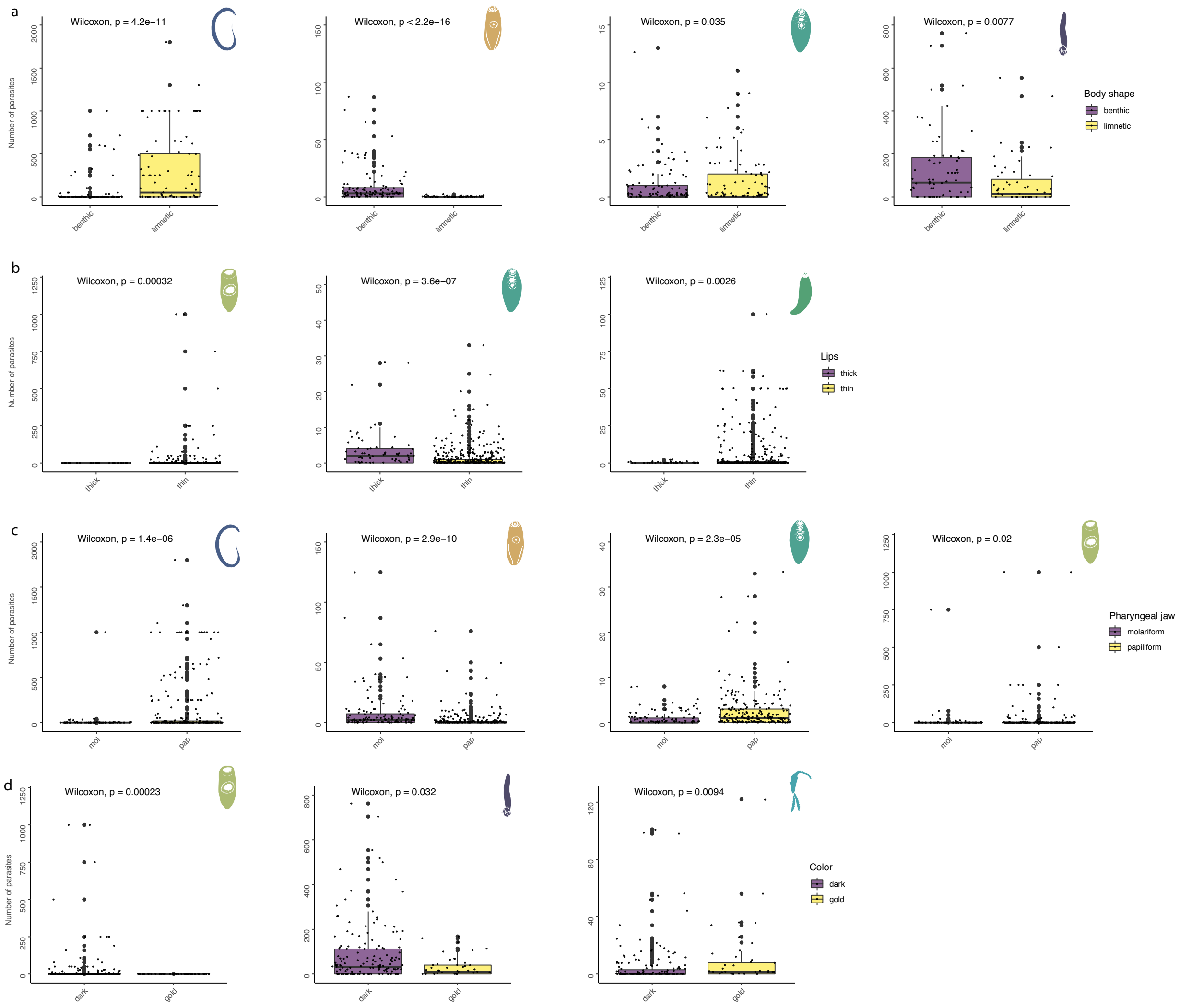

Table S1. GenBank accession number of the 28S region of the taxa used to reconstruct the phylogeny

Species	**GenBank**
Trematoda	
Ascocotyle pindoramensis	KJ094561
Austrodiplostomum ostrowskiae	MF398339
Crassicutis cichlasomae	EU131636
Oligogonotylus manteri	EU662174
Saccocoelioides orosiensis	MG925118
Monogenea	
Sciadicleithrum mexicanum	KY305887
Acanthocephala	
Neoechinorhynchus golvani	KR086244
Nematoda	
Contracaecum spiculigerum	AB189984
Procamallanus barlowi	MW485594
Copepoda	
Acusicola margulisae	MN852851
Branchiura	
Argulus japonicus	KF747852
Outgroup	
Aldersladum sodwanum	MF817947

Table S2. List of parasites infecting the Midas cichlid across the great lakes and crater lakes of Nicaragua.

Parasite taxa	**Adult (A) or larvae (L)**	**Endoparasite (En) or ectoparasite (Ec)**	**Site of infection**
Trematoda			
Ascocotyle pindoramensis	L	Ec	gills
Austrodiplostomum compactum	L	Ec	eye
Crassicutis cichlasomae	A	En	gut
Oligogonotylus manteri	A	En	gut
Saccocoelioides orosiensis	A	En	gut
Saccocoelioides cf. *lamothei*	A	En	gut
Cestoda			
Cichlidocestus janikae	A	En	gut
Schyzocotyle acheilognathi	A	En	gut
Monogenea			
Sciadicleithrum mexicanum	A	Ec	gills
Acanthocephala			
Acanthocephala gen. sp.	L	En	body cavity
Neoechinorrynchus costarricense	A	En	gut
Nematoda			
Contracaecum sp. 1	L	En	body cavity
Contracaecum sp. 2	L	En	body cavity
Contracaecum sp. 3	L	En	body cavity
Goezia sp.	A	En	gut
Procamallanus barlowi	A/L	En	gut
Rhabdochona sp.	L	En	gut
Copepoda			
Acusicola margulisae	A	Ec	gills
Acusicola sp.	A	Ec	gills
Ergasilidae gen. sp.	A	Ec	gills
Lernaeidae gen. sp.	A	Ec	gills
Branchiura			
Argulus sp.	A	Ec	skin/mouth

CAPÍTULO 6. Discusión

Conocer las causas que conducen a la diversificación de los organismos han motivado las preguntas de numerosos biólogos. Desentrañar qué promueve la diversidad es imposible sin el conocimiento de cómo los organismos interactúan entre sí. El parasitismo es sin duda alguna una de las formas de vida más pervasivas en el planeta (Price, 1980), de modo que la interacción dinámica entre hospederos y parásitos podría moldear gran parte de la biodiversidad. Las radiaciones adaptativas explosivas de los cíclidos son un modelo de estudio (Salzburger, 2018), pero prácticamente se desconoce su fauna de parásitos. Este proyecto representa el trabajo más completo hasta la fecha sobre la fauna de parásitos de peces de los lagos de Nicaragua, habiendo profundizado en el estudio de las poblaciones del cíclido de Midas, un ejemplo clásico de repetidas radiaciones adaptativas en simpatría y en paralelo (Barluenga & Meyer, 2004, 2010; Barluenga *et al.*, 2006; Elmer *et al.*, 2010b; a; Elmer & Meyer, 2011; Kautt *et al.*, 2016, 2020). El objetivo principal del estudio fue tratar de determinar la contribución de los parásitos sobre la divergencia del cíclido de Midas.

La fauna de parásitos en los cíclidos de Midas y otros peces de los lagos de Nicaragua

Nuestro inventario de la fauna de parásitos de peces en los lagos de Nicaragua reveló la existencia de 42 taxones de parásitos en 27 especies de peces hospederos, incluyendo 20 especies de cíclidos, 10 de los cuales corresponden al cíclido de Midas. Los parásitos son principalmente helmintos, en su mayoría trematodos y nematodos, todos comunes en cíclidos de la tribu Heroini en México y el resto de Centro América. Los cíclidos son infectados por un subgrupo de 37 taxones de parásitos, de los cuales 22 infectan al cíclido de Midas. Éste alberga una fauna núcleo de parásitos, constituida por ocho taxones que se encuentran de forma constante a lo largo de todos los lagos estudiados. Los parásitos tienen historias de vida contrastantes: transmisión directa o vía trófica, maduran en el pez o en algún ave piscívora o presentan distintos grados de especificidad hospedatoria.

En concordancia con un modelo continente-isla, los lagos grandes Managua y Nicaragua albergan una mayor diversidad de parásitos, mientras que los lagos cráter (‘*las islas’*) más pequeños y recientes, tienen una diversidad menor. Estos datos sugieren que los lagos cráter podrían funcionar como filtros ecológicos que permiten que solo ciertas especies persistan, creando comunidades de parásitos formadas por un subgrupo de las especies presentes en los lagos grandes (‘*el continente’*). A nivel de infracomunidad (conjunto de todas las especies de parásitos dentro de cada pez) la diversidad no sigue el mismo patrón, y la riqueza individual es heterogénea en las poblaciones. En el lago cráter con las poblaciones del cíclido de Midas más antiguas (Apoyo) se encontraron los hospederos con la riqueza de parásitos promedio por individuo más alta, mientras que en uno de los lagos cráter más recientes (Apoyeque) se encontraron los individuos con la menor riqueza.

La fauna de parásitos de peces de agua dulce de Nicaragua

Los grupos de peces más dominantes dentro de los lagos de Nicaragua -cíclidos, poecílidos y carácidos- tienen afinidades neotropicales (Ornelas-García *et al.*, 2008; Říčan *et al.*, 2016; Reznick *et al.*, 2017). Congruentemente, albergan una fauna núcleo de parásitos con origen en Sudamérica que parece haberse dispersado y diversificado junto con sus hospederos (Pérez-Ponce de León & Choudhury, 2005; Choudhury *et al.*, 2017), tras haber experimentado los mismos eventos vicariantes que separaron a sus hospederos (Santacruz *et al.*, 2020a; b). En el caso de los cíclidos, también pudieron adquirir secundariamente parásitos de sus ancestros marinos (Mendoza-Palmero *et al.*, 2017). Conjuntamos nuestro recientemente descrito inventario de parásitos de los peces de agua dulce de Nicaragua con todos los registros previos (de 1976 a 2021), y encontramos más de 100 taxones en las aguas dulces de Nicaragua, y 172 taxones para toda América Central Baja (Nicaragua, Costa Rica y Panamá). Los estudios que han generado estos datos abarcan un esfuerzo de muestreo asimétrico donde sólo 51 de las 114 especies de peces de Nicaragua (~45%) han sido analizadas alguna vez en busca de sus parásitos, mientras que sólo el 21% en Costa Rica y 12% en Panamá, aún cuando en estos dos últimos países la diversidad de peces de agua dulce es más alta. Los datos disponibles indican que en esta región hay 22 taxones endémicos y únicamente seis especies de parásitos adultos ubicuas. Además, corroboramos el componente predominante neotropical de los parásitos de la región. Este resultado destaca el enorme componente inexplorado de los parásitos en una región biogeográfica que moldeó la historia evolutiva de varios linajes de peces (Bermingham & Moritz, 1998).

Ensambles de parásitos divergentes en el cíclido de Midas

El cíclido de Midas es infectado casi por las mismas especies de parásitos (fauna núcleo) en toda su distribución, pero la intensidad de la infección es notablemente contrastante a lo largo de las radiaciones. Los ensambles de parásitos son más similares entre las especies simpátricas que alopátricas, generando ensambles anidados con una firma al lago de procedencia. Los ensambles mantienen el patrón de infección a lo largo de las replicas, es decir, tienen estabilidad temporal. Esto cumple con dos de los tres supuestos propuestos por Karvonen & Seehausen (2012) para considerar una contribución de los parásitos durante la divergencia de sus hospederos. Por lo tanto, estos resultados sugieren que las interacciones hospedero-parásito podrían tener un papel en las radiaciones adaptativas del cíclido de Midas, desde etapas de divergencia incipientes asociadas con la disparidad trófica y adaptaciones locales.

Los ecomorfotipos paralelos del cíclido de Midas replican el patrón de infección cuando se considera por separado cada taxón de parásito, reflejando las preferencias tróficas y ambientales de sus hospederos. Aún más, los paralelismos también se replican en parásitos que no son transmitidos vía trófica y que más bien reflejan el comportamiento social de los hospederos. Por ejemplo, los monogeneos son parásitos transmitidos por vía directa, dependiente de la tasa de contacto entre hospederos, de modo que es de esperar que hospederos más cohesivos tengan mayores abundancias de monogeneos. Congruentemente, las formas bénticas que forman grupos más densos albergaron la mayor abundancia de monogeneos en contraste con las formas limnéticas que tienden a poblaciones menos cohesivas.

El cíclido de Midas es capaz de soportar intensidades de infección muy elevadas, lo que implica bien mayor susceptibilidad o bien mayor tolerancia a los parásitos. Desde la perspectiva del parásito, se puede interpretar como un mayor éxito para infectar a su hospedero. En comparación con los estudios realizados en cíclidos africanos, en los que se han descritos infecciones máximas de 152 nematodos y 59 monogeneos (Gobbin *et al.*, 2020), en el cíclido de Midas encontramos infecciones máximas muy superiores, de más de 750 monogeneos, más de 1000 trematodos adultos o más de 1800 larvas de nematodos en algunos individuos. Estudios previos de parásitos en modelos continente-isla han reportado cambios en los patrones de infección tras una nueva colonización (Jorge *et al.*, 2017; Tomé *et al.*, 2018). Las altas intensidades observadas en los lagos cráter y ausentes en los lagos grandes, pueden ser resultado de un *síndrome de isla*. Según el cual, organismos del continente que colonizan una isla experimentan cambios en la historia de vida, morfología o fisiología (Nieberding *et al.*, 2006).

Coloración y parasitismo

El cíclido de Midas es un grupo policromático con una coloración dominante gris con barras negras denominada normal o ‘*dark*’ y otra más conspicua roja, amarilla o blanca denominada ‘*gold*’. Las poblaciones del cíclido de Midas en los lagos de Nicaragua tienen un promedio de 8% de individuos con coloracion *gold*. El policromatismo se da en la mayoría de las especies y morfotipos descritos en este sistema. El análisis de parásitos revela que los individuos con coloración *gold* están en general infectados por menos parásitos que las formas *dark,* independientemente del tipo ecológico. En muchos organismos la coloración es una señal sexual que refleja el grado de infección parasitaria (Milinski & Bakker, 1990; Hund *et al.*, 2020), y por tanto, una señal honesta de la calidad de un individuo (Johansen *et al.*, 2019). En el cíclido de Midas algunos estudios han demostrado el emparejamiento selectivo en función de la coloración (Barlow, 1983), e incluso diferenciación genética entre formas *dark* y *gold* (Elmer *et al.*, 2009). La tendencia a una menor infección en los individuos de coloración *gold* podría explicarse por su menor frecuencia, diferencias en su comportamiento (Barlow, 1983) y un hábitat distinto a las formas *dark*; aguas más turbias o mas profundas donde es menos visible para los depredadores. Todos estos son factores que podrían influir en las probabilidades de adquirir ciertos parásitos.

Una nueva aproximación para medir la diversidad de los ensambles de parásitos

En este estudio ofrecemos una aproximación alternativa a las medidas clásicas para medir la diversidad, usando un método que considera riqueza, abundancia y relaciones filogenéticas de los parásitos dentro del ensamble. Aún mas, el valor individual de diversidad para cada hospedero se extrapoló a un esquema de muestreo completo, es decir, obteniendo valores de diversidad comparables entre tamaños de muestreo asimétricos. Esto requiere un conocimiento taxonómico fino de la diversidad que compone al ensamble, acompañado de datos moleculares. Con esta metodología generamos datos e inferencias distintas a los obtenidas con índices tradicionales como Shannon-Wiener o Simpson. Por ejemplo, determinamos que el Lago cráter Asososca León tiene los niveles más altos de diversidad filogenética y endemismos respecto a la diversidad regional. Es decir, son ensambles con especies de parásitos más distantes filogenéticamente y con abundancias más homogéneas. Este resultado podría ser la consecuencia de: 1) posibles adquisiciones nuevas de parásitos, 2) divergencia de los parásitos posterior a la colonización y/o, 3) las especies muy cercanas filogenéticamente compiten más y son desplazadas. El conocimiento de la estructura filogenética de una comunidad puede ayudar a entender mejor la formación de los ensambles de parásitos en la naturaleza y cómo las especies de parásitos interactúan dentro de sus ‘*islas*’ de hospederos.

La isla de los parásitos: redes de interacción en parásitos de lagos cráter

El hospedero puede ser visto como una isla para sus parásitos (Coyne, 2011), o en este caso, su ‘*lago cráter’*. Las interacciones interespecíficas entre parásitos son similares a las que ocurren en organismos de vida libre (Pedersen & Fenton, 2007). Las especies que comparten hábitat interaccionan con mayor intensidad, por competencia de espacio u otros recursos (Telfer *et al.*, 2010). Esto se traduce en posibles asociaciones entre los parásitos que comparten un nicho dentro del hospedero. En el caso de los peces, el nicho puede referirse a un órgano o un tejido (Pedersen & Fenton, 2007), como las branquias o el intestino. En nuestro análisis, el estudiar a todos los parásitos que ocurren dentro de cada pez, nos ofrece una oportunidad única de conocer toda la diversidad y abundancia de organismos dentro de ‘*la isla’*, y estudiar cómo interactúan entre sí. Es decir, no tenemos un muestreo de la población, tenemos las poblaciones completas de parásitos interespecíficos. Encontramos que las especies de parásitos que co-ocurren dentro del cíclido de Midas interaccionan de forma positiva y negativa dando lugar a ensambles que no ocurren al azar, y asociaciones que posiblemente facilitan o limitan la presencia de otros parásitos. Estas asociaciones no se replican en las radiaciones paralelas por lo que no son predecibles con relación a su ambiente (el hospedero).

6.1 Conclusiones

Este estudio representa el inventario más completo que se tiene hasta el momento de los parásitos que infectan a los cíclidos en los lagos de Nicaragua, con énfasis en el cíclido de Midas. Este precedente permitió determinar que: 1) existen diferencias en los ensambles de parásitos del cíclido de Midas, 2) que las infecciones se mantienen con la misma dirección estables en el tiempo e, 3) infecciones paralelas a lo largo de todas las radiaciones, sugiriendo que los parásitos tienen un papel importante en la divergencia de sus hospederos. Aunque la idea de una selección mediada por parásitos que puede iniciar o mantener la diferenciación entre poblaciones es intuitiva, se necesita generar más evidencia. Este estudio proporciona la base para investigar las dinámicas hospedero-parásito en mayor detalle, dando lugar a un modelo prometedor para estudiar la interacción hospedero-parásito como promotora de la biodiversidad.

6. 2 Perspectivas y preguntas abiertas

¿Realmente los parásitos podrían contribuir a la especiación en este sistema?

Este primer acercamiento a los parásitos del cíclido de Midas, donde hemos identificado una fauna núcleo de parásitos, ensambles diferenciados, redes de interacción e historias evolutivas no-paralelas entre parásitos, abre una increíble gama de posibilidades para seguir explorando las interacciones hospedero-parásito en este modelo de estudio. El paso lógico para seguir sería ligar la fauna parasitaria con '*el carácter mágico'*; genes del sistema inmune (i.e. MHC). Teóricamente, la variación alélica del MHC podría coincidir con las comunidades de parásitos de una población de hospederos determinada (Meyer *et al.*, 2019). Esto conduce a preguntas como, ¿hay una coevolución entre los genes del MHC y los parásitos?, ¿hay un grupo de alelos del MHC 'optimo'?, ¿existe una combinación de alelos de MHC específico para cada ensamble de parásitos? y/o, ¿se trata de alelos específicos asociados a una especie de parásito? Hasta ahora, lo que sabemos es que el cíclido de Midas alberga una gran variación en genes del MHC (Hoffman *et al.*, 2017). El siguiente paso será determinar si el cambio en las comunidades de parásitos en los hospederos en simpatría juega un papel en la divergencia de señales sexuales. Es decir, si hay un efecto de los parásitos sobre la elección de pareja, donde hospederos más infectados podrían tener menor éxito reproductivo.

¿Cuál es el coste real de los parásitos en la eficacia biológica de los peces?

La fauna núcleo de parásitos presente en todas las poblaciones del cíclido de Midas, es la mejor candidata para evaluar si la tolerancia o susceptibilidad a diferentes parásitos varía entre las poblaciones. Una manera de realizar esta evaluación es medir la respuesta fisiológica a diferentes niveles de infección parasitaria. En el futuro, será importante llevar a cabo experimentos, para estudiar el efecto por separado de cada especie de parásito.

¿Los parásitos en los lagos cráter han experimentado radiaciones adaptativas?

Hasta este momento hemos explorado sólo una pequeña fracción de la información genética de los parásitos, pero se requieren más datos para entender su historia evolutiva. Los candidatos lógicos para un análisis genómico exhaustivo son aquellos parásitos donde encontramos una estructura genética que parece ser moldeada por su aislamiento en los lagos, el monogeneo *Sciadicleithrum mexicanum* y el nematodo *Procamallanus barlowi*. Datos genómicos poblacionales podrían ayudar a plantear una hipótesis más robusta sobre las rutas de colonización de los lagos e historia demográfica, así como explorar el sustrato genómico ligado a su adaptación e interacción con el hospedero.

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ORIGINAL ARTICLE

Incipient genetic divergence or cryptic speciation? *Procamallanus* (Nematoda) in freshwater fishes (*Astyanax*)

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Abstract

Hosts provide the main environmental traits parasites have to deal with, resulting in covariation between both associates at both micro- and macro-evolutionary scales; phylogenetic analyses of highly host-specific parasites have shown that parasite and host phylogeny might be highly congruent, and adaptation of a host species to new environments may lead to concordant changes of their parasites. *Procamallanus* (*Spirocamallanus*) *neocaballeroi* is a highly host-specific parasitic nematode of the Neotropical freshwater fish genus *Astyanax* in Mexico. One of the host species of the nematode is the emblematic Mexican tetra, *A. mexicanus,* which exhibits two contrasting phenotypes, a cave-dwelling morph (with troglomorphic features), and the surface-dwelling morph; other congeneric species inhabit rivers and lakes, and some of them occur in sympatry, displaying trophic specializations. Here, we explored the hypothesis that contrasting environments (surface rivers vs cave rivers), and host morphological divergence (sympatric ecomorphs in a lacustrine environment) might result in the divergence of their parasites, even though the hosts maintain a cohesive genetic structure as the same species. To test the hypothesis, several populations of *Astyanax* spp. were sampled to search for *P.* (*S.*) *neocaballeroi*. The nematode was found in 10 of the 52 sampled sites; two localities corresponded to cave populations. The phylogenetic analysis based on COI sequences yielded three major lineages for *P.* (*S.*) *neocaballeroi*. We found no concordance between the three lineages and the habitat where they occur in *Astyanax mexicanus*, even considering those living in drastic environmental conditions (caves), or between these lineages and lacustrine ecomorphs of *Astyanax aeneus* and *A. caballeroi* occurring in sympatry. Instead, genetic lineages of the nematode exhibit a clearer pattern of host species association and geographical distribution; our results showed that *P.* (*S.*) *neocaballeroi* is experiencing an incipient divergence although the morphological study of lineages shows no conspicuous differences.

KEYWORDS

Astyanax, cryptic species, DNA, nematoda, species delimitation, taxonomy

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Diversity of *Rhabdochona mexicana* (Nematoda: Rhabdochonidae), a parasite of *Astyanax* spp. (Characidae) in Mexico and Guatemala, using mitochondrial and nuclear genes, with the description of a new species

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Abstract

Among fish parasitic nematodes *Rhabdochona* is one of the most speciose genera, with *c.* 100 species. Twelve congeneric species occur in Mexican freshwater fishes, in a region located between the Nearctic and Neotropical biogeographical regions. Host association and biogeographical history have determined the high species richness of *Rhabdochona* in Mexico. One of these species, *Rhabdochona mexicana*, is highly specific to the characid genus *Astyanax*. Characids are a group of freshwater fish with Neotropical affinity. In this paper, we explore the genetic diversity of *R. mexicana* through samples obtained from populations of *Astyanax* spp. across river basins of Mexico and Guatemala. Sequences of one mitochondrial and two ribosomal genes were obtained from 38 individuals and analysed using Maximum Likelihood and Bayesian Inference analysis. Phylogenetic analyses using *cox*1, and a concatenated alignment of 18S + 28S + *cox*1 recovered two genetic lineages. One of them corresponded with *R. mexicana sensu stricto*; this lineage included three reciprocally monophyletic subgroups; the other lineage was highly divergent and represented a putative candidate species. A detailed morphological study was conducted to corroborate the molecular findings. We describe a new species herein and discuss the implications of using molecular tools to increase our knowledge about the diversity of a speciose genus such as *Rhabdochona*.

Introduction

The nematode genus *Rhabdochona* Railliet, 1916 comprises *c.* 100 species of intestinal parasites of freshwater fish in all zoogeographical regions except Antarctica (Moravec, 2010). In the Americas, the genus contains 21 species, although they do not comprise a monophyletic assemblage, and their presence in a wide variety of fish species suggests an evolutionary history with extensive ecological host extension and host-switching events (Mejía-Madrid *et al.*, 2007a, b). Most of the congeneric species are highly host specific, usually at the level of host family (Moravec *et al.*, 2012). In Mexico, 12 species of *Rhabdochona* have been reported as parasites of freshwater fishes, i.e. *R. acuminata* Molin, 1860, *R. ahuehuellensis* Mejía-Madrid and Pérez-Ponce de León, 2003, *R. canadensis* Moravec and Arai, 1971, *R. cascadilla* Wigdor, 1918, *R. guerreroensis* Caspeta-Mandujano, Aguilar-Aguilar and Salgado-Maldonado, 2002, *R. ictaluri* Aguilar-Aguilar, Rosas-Valdez and Pérez-Ponce de León, 2010, *R. kidderi* Pearse, 1936, *R. lichtenfelsi* Sánchez-Álvarez, García-Prieto and Pérez-Ponce de León, 1998, *R. mexicana* Caspeta-Mandujano, Moravec and Salgado-Maldonado, 2000, *R. ovifilamenta* Weller, 1938, *R. salgadoi* Caspeta-Mandujano and Moravec, 2000, and *R. xiphophori* Caspeta-Mandujano, Moravec and Salgado-Maldonado, 2001 (Garrido-Olvera *et al.*, 2006; Pérez-Ponce de León *et al.*, 2009, 2010; Aguilar-Aguilar *et al.*, 2010; Moravec *et al.*, 2012). Seven of the 12 species (58%) are endemic and specific to a particular group of freshwater fish. The presence of the remaining five species of *Rhabdochona* in freshwater fish of Mexico reflects the transitional nature of the area, as the country lies between the Nearctic and Neotropical biogeographical regions (Pérez-Ponce de León, 2003; Pérez-Ponce de León and Choudhury, 2005). For instance, *R. canadensis*, *R. ovifilamenta* and *R. cascadilla* are typically associated with Nearctic freshwater fish groups such as catostomids and cyprinids, and a few others (Hoffman, 1999; Arai and Smith, 2016); meanwhile, *R. kidderi* and *R. acuminata* are usually found in Neotropical fishes such as cichlids, heptapterids and characids (Moravec, 1998).

Rhabdochona mexicana is an endemic and highly host-specific species and is morphologically characterized by having 10 anteriorly directed teeth in the prostom, large spicule

ÁPENDICE

Publicaciones en paralelo al proyecto de doctorado (enero 2018 – enero 2022)

Los artículos publicados como resultado de proyectos derivados o en paralelo a la investigación durante el doctorado se muestran en orden cronológico:

Artículo I

Santacruz A, Ornelas-García CP & Pérez-Ponce de León G. (2020) Diversity of *Rhabdochona mexicana* (Nematoda: Rhabdochonidae), a parasite of *Astyanax* spp. (Characidae) in Mexico and Guatemala, using mitochondrial and nuclear genes, with the description of a new species. *Journal of Helminthology*, 94.

Artículo II

Santacruz A, Ornelas-García CP & Pérez-Ponce de León G. (2020) Incipient genetic divergence or cryptic speciation? *Procamallanus* (Nematoda) in freshwater fishes (*Astyanax*). *Zoologica Scripta,* 49, 768–778.