

Research Paper

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Corresponding author:

F. J. García-De León;
Email: fgarciadl@cibnor.mx

Diversity of trematodes (Platyhelminthes) in Mexico with an assessment of the availability of genetic data for their conservation

Y. Velázquez-Urrieta , V. Mendoza-Portillo  and F. J. García-De León 

Laboratorio de Genética para la Conservación, Centro de Investigaciones Biológicas del Noroeste, Calle IPN #195, La Paz, Baja California Sur, México, C.P. 23096

Abstract

Trematodes are one of the most abundant and diverse groups of platyhelminths. They parasitize all major groups of vertebrates as definitive hosts and therefore play an important role in ecosystem composition. It is estimated that 18,000 to 25,000 species of trematodes exist worldwide, of which 685 have been reported in Mexico. Although this group is an integral part of ecosystems, there are still no studies that highlight the importance of parasites, especially in conservation approaches. Here, we recompiled information on the occurrence and available genetic data of trematodes in Mexico to estimate the specific richness of their representation across the Protected Areas (PAs) and provinces of Mexico. We consulted national and international databases (e.g., GBIF, CONABIO, CNHE-UNAM) and genetic repositories (e.g., GenBank) to generate curated datasets. We obtained 6,780 records that represent 99% of species reported in Mexico (680 species), of which only 10.2% are included in PAs. For genetic data, we found information from five nuclear regions (28S, 18S, ITS1, ITS2 and 5.8S) and two mitochondrial genes (COI and NAD1) for 118 species, of which only 3.5% were associated with PAs. With these results, we provide a spatial distribution of records (occurrence and genetic data) of trematodes present in Mexico and its PAs and identify poorly represented biogeographic provinces (e.g., Sierra Madre del Sur). We also highlight that this is the first study in Mexico to include this group in a conservation approach, and we record valuable information for future studies.

Introduction

Trematodes are cosmopolitan parasites found as adults in all classes of vertebrates, and they represent one of the most species-rich groups of metazoan parasites in the world, with about 18,000 to 25,000 nominal species (Esch *et al.* 2002; Kostadinova and Pérez del Olmo 2014). These parasites have a complex life cycle, with a broad spectrum of host invertebrates and vertebrates from different taxa and ecosystems; therefore, they are particularly interwoven into the food-web and energy flow within ecosystems (Schwelm *et al.* 2021). Parasites have an ancient relationship with their hosts, and there is evidence to support the idea that their elimination in the host may lead to a loss of genetic diversity and immune functions (Altizer *et al.* 2003; Weber *et al.* 2022); in consequence, they are considered excellent indicators of ecosystem functioning. Parasite species, which depend directly on host species for their survival, represent a major regulatory force in ecosystems and a significant component of Earth's biodiversity (Dougherty *et al.* 2016). However, this lifestyle leaves parasites especially vulnerable and under threat of extinction, either as a direct result of factors such as climate change or invasive species or due to extinction of the host (Carlson *et al.* 2020; Cizauskas *et al.* 2017; Lafferty *et al.* 2012).

Molecular data of parasites have proven to be a powerful tool for species characterization and delimitation (Blasco-Costa *et al.* 2016; Pérez-Ponce de León and Hernández-Mena 2019; Poulin and Presswell 2016) and for reconstructing historical population size, assessing genetic diversity, and understanding host ecology (Crellen *et al.* 2016; Whiteman and Parker 2005). However, parasite genetic and genomic data have not been employed in conservation assessments due to limited genetic resources and the difficulty of applying conservation concepts to parasite species (Carlson *et al.* 2020). Although parasites are among the most abundant and diverse organisms and form an integral part of the biosphere and ecosystems (Dobson *et al.* 2008; Poulin and Morand 2000; Rubio-Godoy and Pérez-Ponce de León 2023), most wildlife species remain understudied, underfunded, and underappreciated despite a growing body of literature highlighting the importance of parasite-inclusive conservation (Dougherty *et al.* 2016).

Human activities have disrupted much of the planet's natural areas, including terrestrial, ocean, and freshwater habitats (Birk *et al.* 2020; Halpern *et al.* 2008; Sanderson *et al.* 2002). Recent

studies have shown a substantial decline in biodiversity, estimated at 5.4–6.5% since industrialization, mainly due to loss, fragmentation, overexploitation, and destruction of habitat, and other human activities (Exposito-Alonso *et al.* 2022; Leigh *et al.* 2019). Therefore, Protected Areas (PAs) are becoming increasingly important as the only alternative for the conservation and protection of ecosystems and as refuges for wildlife. In general, knowledge of trematodes and other parasites in natural systems is often limited, especially for PAs, which are usually not easily accessible due to restrictions and protective measures. However, to fully evaluate the role of parasite genetic diversity for the conservation of species and ecosystems, including natural protected areas, baseline data are needed. The main aim of this study is to compile information on trematode diversity in Mexico and associated genetic data and analyse the availability of this information for the country's protected areas and biogeographic provinces.

Materials and methods

Information about the trematodes of Mexico (records and metadata) was compiled from electronic public databases such as GBIF (<https://www.gbif.org/es/>, accessed September 29, 2023), CONABIO (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, <https://www.gob.mx/conabio>, accessed April 24, 2024), and UNIBIO (<http://unibio.unam.mx/>), and an exhaustive literature search. This information was captured in Microsoft Access 2010. For each dataset, we included information about the taxonomic identifier, host (species, role: intermediate or definitive), environment (hydrologic and marine regions, vegetation), data record (locality, state, geographical coordinates), distribution in protected areas, gene(s) amplified, and bibliographic reference(s) (first author, publication year, title and doi of publication). To validate the records, we manually checked for record duplicates, incomplete records (without a locality, coordinates or bibliographic reference), and taxa identified only to the genus or family level. Those records were excluded from the dataset. We also excluded records that identified localities only at the level of a general region, state, or province. Some records providing locality but missing geographical coordinates (latitude and longitude) were checked through GEOlocate (www.geo-locate.org, accessed December 19, 2024) and GeoNames (<https://www.geonames.org>, accessed January 20, 2024). For the taxonomic validation, we followed the trematode species' nomenclature and classification from *Keys to the Trematoda* (Bray *et al.* 2008; Gibson *et al.* 2002; Jones *et al.* 2005). We built the first database with all records of trematodes ('record dataset'). The second database was built for species with genetic data; that information was gathered from an exhaustive review of publications and by screening public repositories such as GenBank (<https://www.ncbi.nlm.nih.gov/genbank>, accessed March 10, 2024) and BOLD (<https://boldsystems.org/>, accessed April 20, 2024) ('genetic dataset'). The following genetic data were downloaded for trematodes: nuclear genes 5.8S, 28S (LSU), and 18S (SSU); internal transcribed spacers ITS1 and ITS2; and mitochondrial DNA genes COI and NAD1, for records previously compiled in the first database. These molecular markers have been sequenced for trematodes in Mexico (see Pérez-Ponce de León and Hernández-Mena 2019). The third database contained trematode records with genetic data corresponding to the polygons of PAs of Mexico ('PA dataset'). The information (area number, maps, polygons/point ratio, etc.) for Mexico's PAs was downloaded from the following websites: Comisión Nacional de Áreas Naturales Protegidas (CONANP, www.gob.mx/conanp, accessed April 30, 2024), CONABIO (<https://www.gob.mx/conabio>, accessed

April 30, 2024), and Protected Planet (<https://www.protectedplanet.net/country/MEX>, accessed April 30, 2024).

Dataset availability analyses

To assess the availability and trends of records and genetic data for trematodes from Mexico, we mapped the localities of the record dataset and genetic dataset using QGIS v. 3.36 (www.qgis.org). We took into consideration a 1-km buffer around the coordinates to account for uncertainty due to georeferencing procedures, consistent with the median coordinate uncertainty according to Crandall *et al.* (2023). To estimate the proportion of trematode species in our datasets in relation to the total number of known species in Mexico, we consulted the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). This collection is Mexico's main parasite repository and holds the most complete and up-to-date information on trematodes.

To demonstrate any association between the record and genetic datasets, and biogeographic and hydrographic predictor variables, the records and genetic information were associated in a 1x1° grid, then the number of species was counted in each quadrant. Pearson's correlation and linear regression were evaluated considering the species and family taxonomic level of records and genetic points in the R Stats Package (R Core Team 2023). For biogeographic association, we used the shapefile of the biogeographic provinces of Mexico provided by Morrone *et al.* (2017), considering two levels (provinces and regions) and the main rivers reported in the hydrology shapefile of the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO 2024).

Results

Trematode species richness

A total of 12,606 trematode records were collected. After excluding duplicate records and those with incomplete data, we retrieved 6,780 records (1,302 of which we georeferenced ourselves). Some studies (3,365) did not accurately specify the locality, and these records were not georeferenced; some records (1,695) included taxa identified only to the genus or family level and therefore were excluded from the analyses because they may pertain to more than one species or be a synonym of an already-named species. Trematodes recorded in Mexico in this study are represented by 680 species (identified to species level) included in 320 genera and 82 families; most records correspond to adult trematodes and a few to larval stages, as sporocysts, cercariae and metacercariae. The majority of trematode records correspond to the vertebrate host, in descending order of importance: fishes, amphibians, reptiles, mammals, and birds. For the first time, records of the trematodes of invertebrate hosts (snails and insects) were integrated.

The largest number of records correspond to specimens denominated *Posthodiplostomum minimum* (MacCallum, 1921) (total: 672), recorded principally in fishes (second intermediate host) and some in birds (definitive host). These are followed by *Oligogonotylus manteri* (Watson, 1976) (total: 479), recorded in fishes (definitive host); then by *Langeronia macrocirra* (= *Loxogenes macrocirra*) (Caballero and Bravo-Hollis, 1949) (total: 195), reported in freshwater snails (first intermediate host), insects (second intermediate host), and amphibians (definitive host);

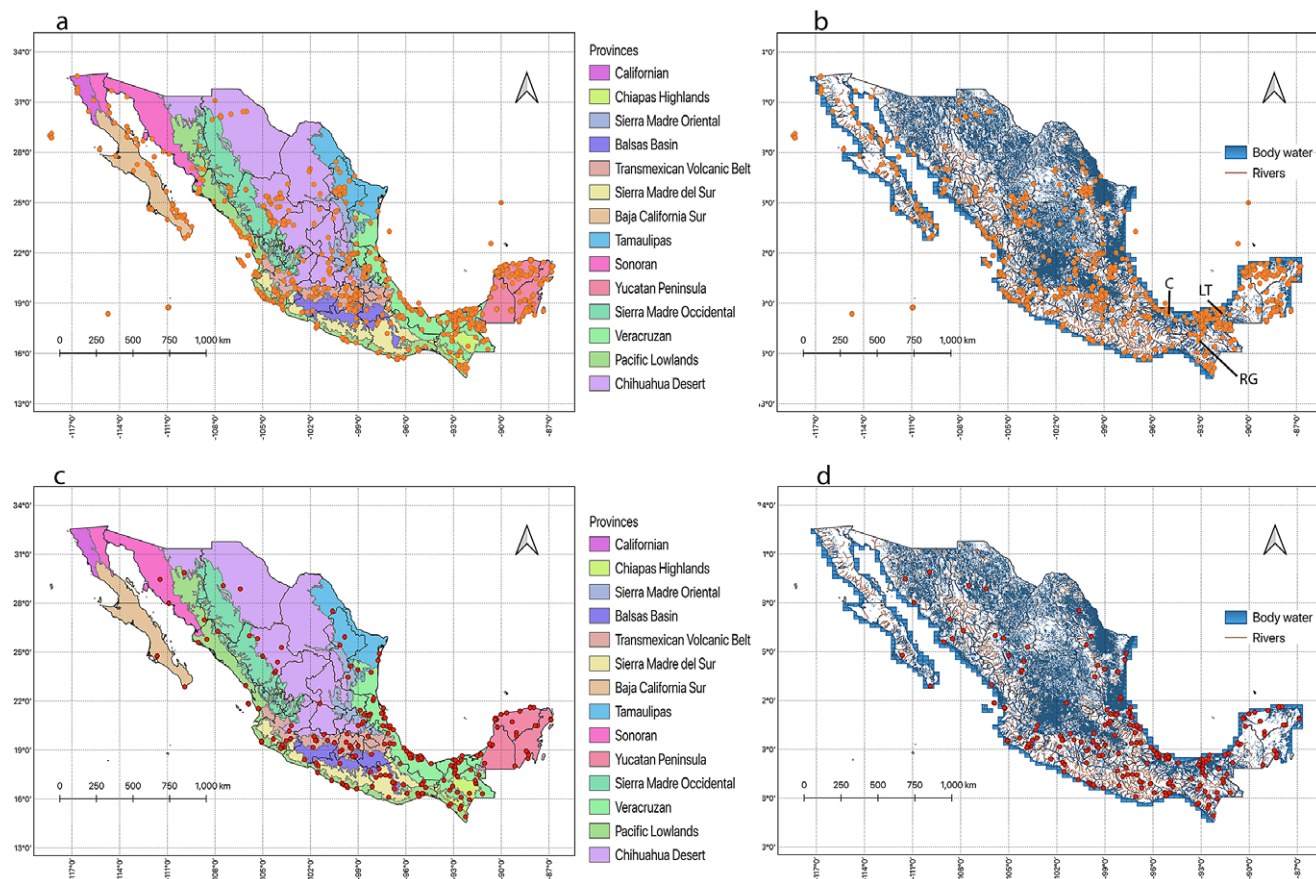


Figure 1. Maps of Mexico showing the distribution of available information on trematodes, Mexican provinces, and the Mexican hydrological system (rivers and bodies of water). (a) General records (presences) and Mexican provinces; (b) General records (presences) and the Mexican hydrological system; (c) Genetic records and Mexican provinces; (d) Genetic records and the Mexican hydrological system.

and lastly, *Crassicutis cichlasomae* (Manter, 1936) (total: 149), recorded in fishes (definitive host).

Based on the geographical coordinates, trematode records in Mexico exhibit an asymmetric distribution, as shown in Figure 1a. The number of records per site ranges from 1 to 96; therefore, numerous points in the map correspond to a cluster of records. The map shows some zones (central and southeast) with a high number of records, while others (northeast) have few records; some areas even remain unexplored, leaving large gaps in information. The provinces with the most trematode records were the Veracruz (2280), Yucatán Peninsula (1065), and Pacific Lowlands (733) [Figure 1a], while Sierra Madre del Sur province has the fewest records (15). The majority of records correspond to trematode parasites from fishes and amphibians; indeed, most of these records are associated with important lagoons and rivers (e.g., Laguna Términos, Lago de Catemaco, and Río Grijalva), while ocean records are scarce (Figure 1b).

Availability of genetic data

Very few records of trematode species are supported by genetic data for at least one DNA sequence in Mexico. The information gathered shows that only 1,155 records presented genetic data, representing 17.03% of the total records for the country. We gathered a total of 2,064 sequences for mitochondrial genes (COI, NAD1), 2,759 sequences for nuclear genes (28S/LSU,

18S/SSU), and 905 sequences of internal transcribed spacer + 5.8S (Table 1). Genetic information has been generated for at least one gene for only 118 of the species reported in this work, equivalent to 17.35% of species reported in this study. The species with most genetic data were *C. cichlasomae* (368), *Clinostomum tataxumui* (Serenó-Uribe, Pinacho-Pinacho, García-Varela and Pérez-Ponce de León, 2013) (total: 189), *L. macrocirra* (total: 80), and *Austrodiplostomum compactum* (Lutz, 1928) [total: 60]. The

Table 1. Genetic data of trematodes from Mexico, including genes, sequence numbers, and species numbers.

| Gene | N. of sequences | N. of species |
|--|-----------------|---------------|
| Mitochondrial | | |
| Cytochrome oxidase subunit 1 (COI) | 1960 | 70 |
| Nicotinamide adenine dinucleotide dehydrogenase subunit 1 (NAD1) | 104 | 3 |
| Nuclear | | |
| 28S ribosomal RNA/ LSU | 2120 | 112 |
| 18S ribosomal RNA / SSU | 621 | 60 |
| 5.8S ribosomal RNA | 18 | 10 |
| ITS1+5.8S+ITS2, ITS1, ITS2 | 905 | 111 |

most sequenced molecular marker was the nuclear 28S gene, although the mitochondrial gene COI exhibited the highest number of sequences per species, while the genes NAD1 and 5.8S had the lowest number of sequences.

Based on the geographical coordinates of the sequences, the genetic data available for trematodes from Mexico is sparsely and unevenly distributed across the country. Figure 1c represents the distribution of different trematode species with genetic data; each point represents a record with at least one genetic sequence. The map shows that the greatest number of trematode records with genetic data were found in southeastern Mexico, whereas the north of the country exhibits very few records with this information. A province-level analysis found that the three with the most genetic records were the Veracruz (total: 391), Pacific Lowlands (total: 63), and Transmexican Volcanic Belt (total: 26) provinces (Figure 1c), while the Californian province had no genetic records for trematodes. Most records correspond to trematode parasites of fishes and amphibians; many are associated with important lagoons and rivers (Figure 1d).

Trematode richness inside PAs

Our analysis considered 843 PAs, of which only 86 (10.2%) presented at least one record of trematodes. Of these, 83 were terrestrial areas and three were marine protected areas. Overall, 444 records were found for trematodes within the 86 PAs, represented by 212 species from 132 genera and 55 families. The largest number of records were found in the PAs of Los Tuxtlas Biosphere Reserve (T, 241) in Veracruz, Laguna de Términos (LT, 102) in Campeche, and Pantanos de Centla (PC, 101) in Tabasco (Figure 2a). The most widely distributed trematode species within PAs were the diplostomid *P. minimum* and the megaperid *C. cichlasomae*. Genetic data for trematodes within the Mexican PAs was even more limited; only 3.5% (30) of PAs presented genetic data (Figure 2b). In total, 117 trematode records with genetic information were localized inside Mexican PAs, represented by 67 species. The PA with the largest number of genetic data records was Los Tuxtlas Biosphere Reserve.

Dataset availability analyses

Pearson's correlation analysis did not find a clear pattern between number of species or families by quadrant and biogeographic or hydrographic regionalization ($r < 0.36$; see supplementary Figure S1), while linear regression showed a weak but significant relationship only for records of species and genetic sequences ($r^2 = 0.345$; see supplementary Figure S2, supplementary table S1). For the biogeographic predictor variable at province level, the relationship was $r^2 = 0.4059$, higher than for a region level ($r^2 = 0.3655$, Supplementary Figure S2 b, c); although the Neotropical region presents the most records of species and genetic information, a non-significant relationship was found (Figure S2c, Table S1c). The hydrographic predictor shows a stronger and significant relationship between genetic and species records ($r^2 = 0.862$, Figure S2d), where the most represented rivers were Río Grijalva and Río Tuxpan ($p = 0.001$, Table S1), located in the southeast of Mexico.

Discussion

Our study has revealed that while trematodes are well represented in taxonomic records in Mexico, genetic data are still scarce for most taxa and areas, principally inside of PAs. We acknowledge that sampling hosts within protected areas is challenging, since in most instances, invasive sampling is required to obtain parasites; however, this can provide valuable information about parasites and hosts, and a general overview of diversity in these important regions for biodiversity conservation. Trematodes are the most well-studied group of helminths in Mexico, with the largest number of known species, and they have been studied in different vertebrate host groups. In 2001, Pérez-Ponce de León (2001) reported 503 species of Digenea in wildlife vertebrates for Mexico; subsequently, Pérez-Ponce de León *et al.* (2007) reported 528 species, and García-Prieto *et al.* (2014) reported 544 trematode species as of 2014. As of May 2024, we reported 680 species based on information collected in this study, while 685 species have been recorded according to data from CNHE-UNAM (García-Prieto, personal communication), including only taxa identified to a specific level, because records determined to genus level may be synonyms of already-

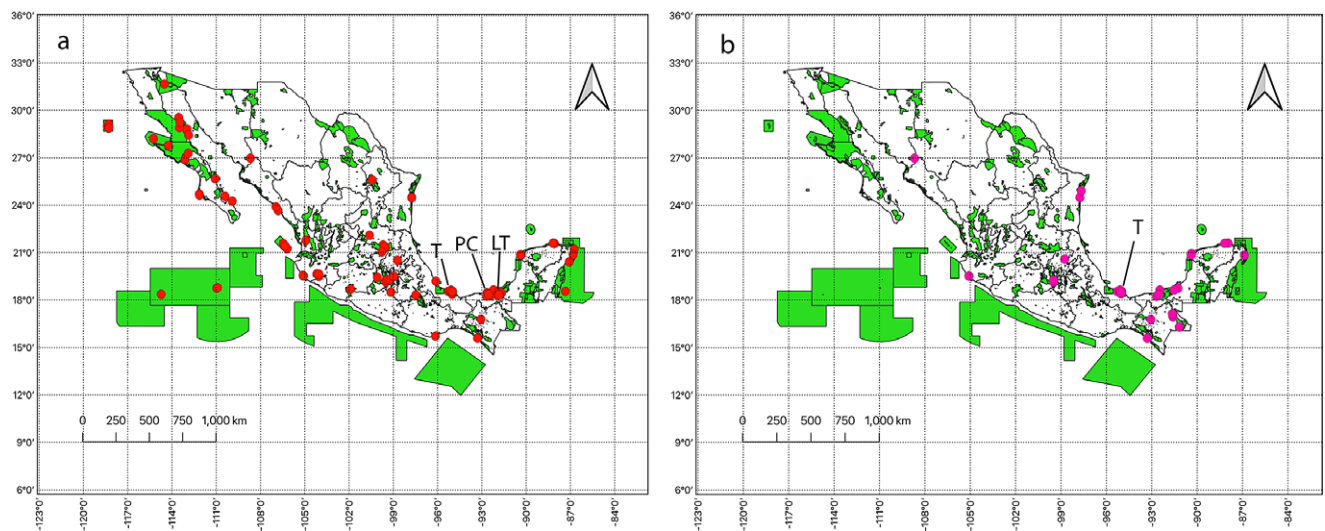


Figure 2. Map of Mexico showing the distribution of trematodes within protected areas marked in green. (a) Taxonomic records (presences); (b) Records with genetic data. T= Los Tuxtlas, PC= Pantanos de Centla, and LT= Laguna de Términos.

named species or may pertain to more than one species. It follows that 141 species have been recorded in the past 10 years, approximately 14 species per year, pointing to significant advances in knowledge of the diversity of trematodes in Mexico. In the last few years, other hosts have been explored (e.g., snails), and molecular tools have been implemented to identify the larval stage and establish their life cycle (see Velázquez-Urrieta and Pérez-Ponce de León 2021a). Nevertheless, the inventory of the trematode fauna is still far from complete in Mexico because some regions and groups have been scarcely explored. Therefore, it is necessary to develop projects focused on these areas of Mexico to improve knowledge of trematode diversity.

Genetic data available

The scarcity of genetic data for trematodes in Mexico (available only for 17.35% of species; see results) is due to several factors, but principally a lack of interest by the government and other agencies in funding projects focused on parasite inventories, resulting in a dearth of resources for generating genetic data. However, this deficiency can be overcome through collaboration among researchers, especially partnerships with universities or other institutions that already have equipment, materials, and supplies for the generation of genetic data (Cabrera-Guzmán *et al.* 2021). Only then will it become possible to gradually close the gap between taxonomic records and genetic data for this group of parasites. We also found that some researchers do not upload genetic data generated to public databases (GenBank, Bold), resulting in the loss of valuable information for future analyses. In Mexico, the parasites of wildlife have been studied from a taxonomic approach since the 1930s, beginning with work by Sokoloff and Caballero (1932), and since then have been the object of frequent study principally by Mexican researchers. While the implementation of molecular tools remains at a relatively early stage, the first works including genetic data on trematodes in Mexico date back to the 1990s (see Campos *et al.* 1998; León-Règagnon *et al.* 1999). Although these powerful tools have been employed by some researchers in inventories of parasites, such work remains scarce compared to studies with a taxonomic approach. Genetic data generation and availability are essential and highly useful in research approaches, including for species identification (see Antil *et al.* 2023; Hebert *et al.* 2004), assessments of biodiversity conservation (see Andrello *et al.* 2022), and the elucidation of trematode life cycles (Velázquez-Urrieta and Pérez-Ponce de León 2021b). This work showed that the mitochondrial gene COI has been generated for few trematode species in Mexico; therefore, it has a lower number of sequences in comparison to the nuclear rDNA 28S, which limits its use as a barcode in most species in this group. However, we found that some species, such as *C. tataxumui* and *L. macrocirra*, have large numbers of COI sequences from different stages, specimens, localities, and hosts, which allows various phylogenetic and phylogeographic inferences to be made about these species; in other cases, it has allowed the identification of new species (see Velázquez-Urrieta and Pérez-Ponce de León 2021b) or links between different stages of the same species (see Velázquez-Urrieta and Pérez-Ponce de León 2022). The high availability of rDNA 28S sequences per species has enabled the phylogenetic classification of Digenea (see Pérez-Ponce de León and Hernández-Mena 2019). The 28S sequences are also very useful for determining the phylogenetic position of species at the family, genus, and, in some cases, species level, so many researchers have chosen to generate this gene more frequently than the COI gene and others. Including this information in taxonomic

studies makes it possible to implement an integrative taxonomic approach that reduces the substantial gaps in parasite studies (Nadler and Pérez-Ponce de León 2011). The paucity of genetic data for trematodes from Mexico limits our knowledge and the implementation of studies of diversity in this parasite group, mainly contemporary and historical demographic studies that allow determining the effects of fragmentation and/or climate change on coevolution with their hosts. However, we hope that future studies and collaborations between researchers can generate more and better genetic information about trematodes in this country.

Geographic distribution of the trematode records

In Mexico, taxonomic and genetic trematode records show an asymmetric distribution; they are most heavily concentrated in the southeast of the country, while the north has few records. This same asymmetry was found in the biogeographic provinces of Mexico; the highest number of taxonomic records were from the Veracruz, Yucatán Peninsula, and Pacific Lowlands provinces, while the greatest number of genetic records were found in the Veracruz, Pacific Lowlands, and Transmexican Volcanic Belt provinces (Figure 1a, c). In both cases, the Veracruz province exhibited the most records, principally the region of Los Tuxtlas (31%), home to the Los Tuxtlas Biosphere Reserve (Figure 2b). Cabrera-Guzmán *et al.* (2021) found the same pattern for helminthological records from amphibians (with the exception of the Yucatán Peninsula province) and attributed the high number of helminth records in the Veracruz province to the various helminth projects undertaken at the Los Tuxtlas Tropical Biology Station from Universidad Nacional Autónoma de México (EBTLT-UNAM). This field station plays an important role, but we believe this can also be explained by the fact that this region is the last remnant of the rainforest in Mexico and is the area most threatened by livestock farming, degradation, and deforestation. This has led to research to gain insight into the diversity of this important ecosystem (see Velázquez-Urrieta and Pérez-Ponce de León 2021). The high concentration of trematodes recorded in some regions and provinces in Mexico is due to the easy accessibility of those areas for research. However, this has resulted in an asymmetric distribution of records, which are highly concentrated in some areas but sparse in others. To gain a more thorough understanding of trematode diversity in this country, it is necessary to explore other areas with few or no trematode records, such as the Sierra Madre del Sur or the Sierra Madre Occidental provinces, where records are absent or scarce (Figure 1a, c). Furthermore, we note that the majority of trematode records are associated with important freshwater bodies and rivers (e.g., Lago de Catemaco, Veracruz; Laguna Santa Anita, Tabasco; Río Papaloapan and Río Usumacinta) (Figure 1b, d), with high record-genetic data correlation in Río Grijalva and Río Tuxpan (see supplementary Figure S1 and Figure S2d). These patterns are mainly due to a wealth of research into trematode parasites of freshwater fishes and amphibians (e.g., Pérez-Ponce de León *et al.* 2000; Salgado-Maldonado *et al.* 2005); our study found 46% of records pertained to freshwater fishes, 17% to amphibians, and the remaining 37% to other hosts. This shows that other host groups have been relegated in the study of trematode diversity.

Protected areas

In Mexico, like other parts of the world, many species of hosts (vertebrates and invertebrates) have become extinct in the wild (due to habitat degradation, pollution, and climate change), and with

them, so have their parasites. Parasite data may contribute to a conservation plan for parasites and a particular group of vertebrates or invertebrates (host). Therefore, complete knowledge of the parasites found in the host population targeted by a given conservation strategy makes it possible to determine the fate of this population and evaluate the possibility of survival within a scenario of emerging diseases (Pérez-Ponce de León *et al.* 2007) and climate change.

In Mexico, knowledge of parasite groups in natural systems is still limited, especially in protected areas. These areas are accessible for many biologists, but less so for parasitologists due to restrictions and protective measures. Therefore, assessments of parasite biodiversity in such areas encounter various obstacles and are inevitably associated with administrative burdens (Schwelm *et al.* 2021). We showed that only 11% of Mexican PAs contained records of at least one trematode species in some intermediate or definitive host; however, this proportion fell sharply for records with genetic data (see results), with only 4.6% of PAs exhibiting genetic records (Figure 2a, b). We found that the number of genetic data points was relatively higher in the Los Tuxtlas Biosphere Reserve (tropical rain forest); this can be attributed to the presence of the EBTLT-UNAM, where molecular studies have been conducted on trematode species (Velázquez-Urrieta and Pérez-Ponce de León 2020, 2021a, 2022). Knowledge of trematode diversity in protected areas is fundamental because it may represent the best approximation of the natural status of the parasites in natural systems. This can be used as a basis to assess changes in trematode and host community composition in heavily modified ecosystems (Schwelm *et al.* 2021). Therefore, parasitologists need to implement new strategies that are not invasive for hosts and that allow the assessment of parasite diversity in protected areas with the least possible impact (e.g., Velázquez-Urrieta and Pérez-Ponce de León 2021a). Also, governmental restrictions and protective measures should be conducive to research by parasitologists. Without this basic information on trematodes, it will be difficult to predict whether and how parasites and their hosts will react to the ongoing anthropogenic-driven habitat alterations and how these reactions will affect ecosystem processes in the face of global climate change (Schwelm *et al.* 2021). Therefore, research in PAs is essential to evaluate the role of parasites in the conservation of species and ecosystems and to provide baseline data for future research.

Future trematode research

Information about trematode diversity in Mexico remains limited, and some regions are unexplored. In this context, there is a need for researchers to implement strategies and methods that allow them to generate more information, and more thorough data, in the shortest amount of time possible. Therefore, we recommend that researchers consider the following aspects in future studies: 1) identify regions, areas, and states where records are limited and implement projects accordingly; 2) include all possible metadata in future records and research; 3) include the molecular characterization of specimens in their research; 4) upload the genetic data generated to public databases, such as GenBank or Bold; and 5) in PAs, undertake projects on parasite diversity with non-invasive methods – for example, by using intermediate hosts, specifically snails, which provide an overview of the diversity of trematodes without a need to sacrifice the host (see Schwelm *et al.* 2021; Velázquez-Urrieta and Pérez-Ponce de León 2021a).

In conclusion, we identified geographical, taxonomic, and genetic gaps in trematode information in Mexico. While taxonomic

studies are fundamental, they need to be complemented with other information – for example, on genetic, ecological, and phylogenetic aspects of trematodes. We emphasize the need for further research with a molecular and taxonomic approach, which is essential for better knowledge of trematode diversity. We also encourage researchers to conduct studies with non-invasive methods in protected areas and other areas of Mexico, while remaining open to collaborations with other researchers for the generation of genetic data, as comprehensive knowledge of parasites can provide valuable insights into biodiversity across the various ecosystems and PAs in Mexico.

Supplementary material. The supplementary material for this article can be found at <http://doi.org/10.1017/S0022149X24000907>.

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