



ORIGINAL ARTICLE

Differential diagnosis of human *Entamoeba* infections: Morphological and molecular characterization of new isolates in Argentina



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KEYWORDS

Entamoeba;
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Abstract *Entamoeba* infections occur worldwide, with higher frequency in countries of low socioeconomic status and poor public health. Since *Entamoeba histolytica* has long been recognized as the only pathogenic species, making a differential diagnosis of other morphologically identical *Entamoeba* is important. This study aimed to determine the prevalence of *Entamoeba* species in two populations from Argentina, make a differential diagnosis by PCR and characterize *Entamoeba* isolates at the SSU rRNA gene. A total of 493 serial fecal samples were obtained from individuals in the provinces of Buenos Aires (n = 210) and Misiones (n = 283). Samples were examined by conventional methods (formalin–ethyl acetate and Willis flotation) and specific PCRs to differentiate *Entamoeba* species. *Entamoeba* isolates were characterized by sequencing a fragment of the SSU rRNA gene. The overall prevalence of *Entamoeba* infection was 12.4%, being more prevalent in Buenos Aires than in Misiones (14.8% vs. 10.6%). A case of *E. histolytica* confirmed by PCR and sequence analysis was reported for the first time in Buenos Aires. Moreover, new genetic data on *Entamoeba coli* and *Entamoeba dispar* were recorded. The phylogenetic analysis revealed a congruence between morphological characteristics and SSU rRNA gene sequences. This study increases the amount of information on the distribution of these species in Argentina and the region of the Americas.

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PALABRAS CLAVE

Entamoeba;
Prevalencia;
Argentina

Diagnóstico diferencial de infecciones humanas por *Entamoeba*: caracterización morfológica y molecular de nuevos aislados en Argentina

Resumen Las infecciones por *Entamoeba* se producen en todo el mundo, con mayor frecuencia en países de bajo nivel socioeconómico y salud pública deficiente. Dado que se ha reconocido a *Entamoeba histolytica* como la única especie patógena del género, es importante realizar un diagnóstico diferencial respecto de otras especies de *Entamoeba* morfológicamente idénticas. Este estudio tuvo como objetivos determinar la prevalencia de especies de *Entamoeba* en 2 poblaciones de Argentina, realizar su diagnóstico diferencial por PCR y caracterizar los aislados de *Entamoeba* secuenciando un fragmento del gen SSU ARNr. Se obtuvieron 493 muestras fecales seriadas de individuos de las provincias de Buenos Aires (n = 210) y Misiones (n = 283). Las muestras se examinaron por métodos convencionales (sedimentación de formalina-etil acetato y flotación de Willis) y mediante PCR específicas para diferenciar especies de *Entamoeba*. Los aislamientos de *Entamoeba* se caracterizaron por secuenciar un fragmento del gen SSU ARNr. La prevalencia general de la infección por *Entamoeba* fue del 12,4% y fue mayor en Buenos Aires que en Misiones (14,8 vs. 10,6%). Se informó por primera vez un caso de *Entamoeba histolytica* en Buenos Aires, confirmado por PCR y análisis de secuencia. Además, se registraron nuevos datos genéticos sobre *Entamoeba coli* y *Entamoeba dispar*. El análisis filogenético reveló una congruencia entre las características morfológicas y las secuencias del gen SSU ARNr. A través de este estudio, hemos sumado información acerca de la distribución de estas especies en nuestro país y en la región de las Américas.

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Introduction

The protozoan species *Entamoeba dispar*, *Entamoeba histolytica*, *Entamoeba moshkovskii*, and *Entamoeba bangladeshi* are collectively referred to as the *Entamoeba* complex because they are morphologically identical¹. Among these species, *E. histolytica* is the causative agent of amebiasis in humans, which is associated with intestinal and/or extra-intestinal manifestations. Infection typically initiates with the ingestion of mature cysts found in food, water, or hands contaminated with stool^{5,21}. Although amebiasis is recognized as a neglected emerging disease, most cases are asymptomatic. Individuals with symptomatic amebiasis often suffer from amebic colitis and amebic liver abscess²⁴. Symptoms of amebic colitis can range from mild diarrhea to severe dysentery, with abdominal pain and watery or bloody diarrhea¹⁸. Meanwhile, severe chronic infections may lead to further complications such as peritonitis, perforations, and the formation of amebic granulomas²¹.

On the other hand, the pathogenicity of *E. moshkovskii* and *E. dispar* remains unclear⁷. Recent studies have shown that *E. moshkovskii* can cause gastrointestinal disorders^{13,33}. Furthermore, previous studies have indicated an association between *E. dispar* and clinical symptoms^{19,41}. Meanwhile, *E. bangladeshi* has been isolated from fecal samples of both asymptomatic children and those experiencing diarrhea¹⁴.

Traditionally, amebic infections have been diagnosed by microscopy; however, this method relies on the observers' experience and cannot distinguish among the *Entamoeba* complex species. On the other hand, many diagnostic tools based on stool polymerase chain reaction (PCR) have the

highest sensitivity in distinguishing species³⁴. In this sense, the small subunit rRNA (SSU rDNA) gene has been widely used to analyze phylogenetic relationships among eukaryotic organisms and, mainly, to detect *Entamoeba* species in stool samples³⁶. *Entamoeba* SSU rRNA can be found in multiple copies of extrachromosomal plasmids and is relatively fast-evolving; hence, providing sufficient resolution to differentiate *Entamoeba* taxa¹².

Non-pathogenic amebae, such as *Entamoeba coli*, *Entamoeba polecki*, *Entamoeba hartmanni*, *Endolimax nana*, and *Iodamoeba bütschlii* may also be confused with *E. histolytica* in diagnostic investigations⁵. Therefore, an accurate diagnostic strategy is needed to avoid misdiagnosis and wrong treatment²⁴.

In the region of the Americas, *E. coli* was determined as the most prevalent and widely distributed ameba by conventional methods (e.g., direct smear and formalin–ethyl concentration), followed by the *Entamoeba* complex. However, more studies based on molecular techniques are needed to corroborate if conventional methods overestimate the prevalence³¹.

In Argentina, there are limited studies regarding the differential prevalence of *Entamoeba* species. In the last three decades, most of the prevalence studies were mainly based on microscopy and reported *E. coli* as the most frequent species (Fig. 1). In addition, most of these studies reported the diagnosis as *Entamoeba* complex, which was usually determined by microscopy²⁰. Only one research performed an ELISA test and found a seroprevalence of 28% (21/75) of *E. histolytica* in a native community from the province of Santa Fe². This protozoan parasite was also detected in Buenos Aires by microscopic examination with a low prevalence

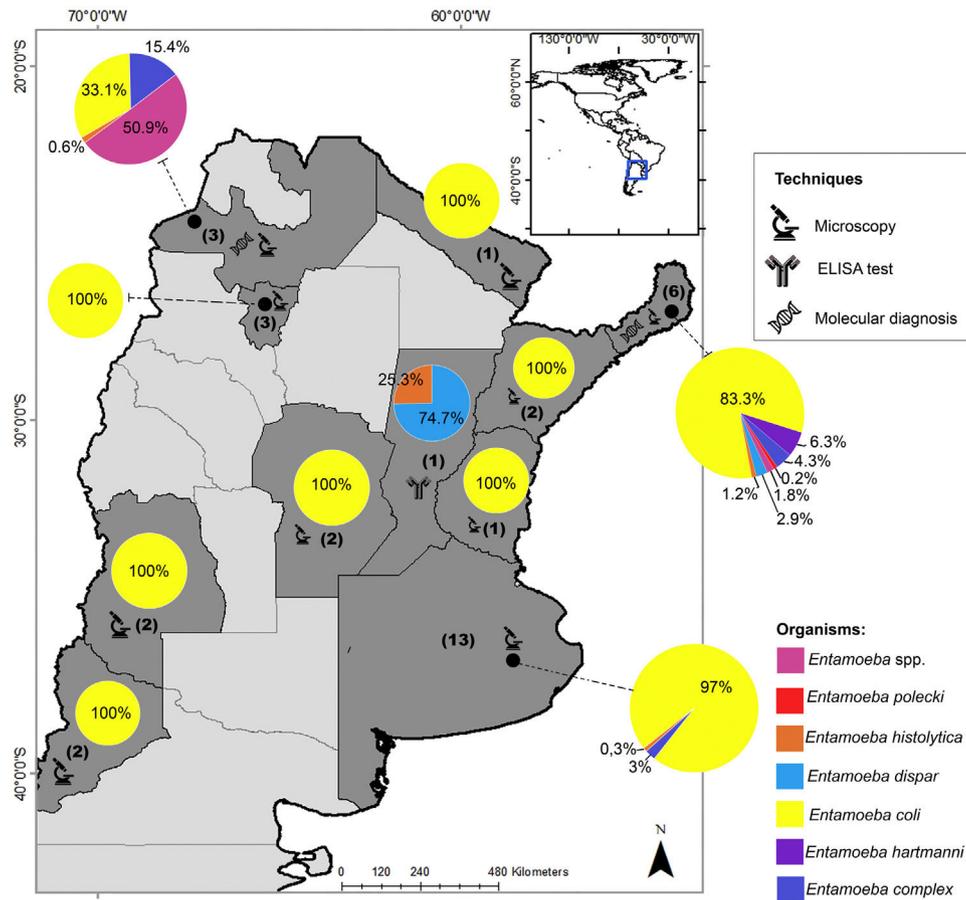


Figure 1 Geographical distribution of *Entamoeba* spp. detected by studies performed in Argentina in the last three decades. Pie charts represent the proportion in which each *Entamoeba* species was detected. The number of studies that examined the prevalence of amebae in each province of Argentina is shown in brackets. Techniques performed for diagnosis are also represented. The map was drawn up based on [Supplementary Table 1](#). Maps were performed using QGIS version 3.12 (Quantum GIS Development Team, 2020).

(0.9%; 2/210)⁹. *E. histolytica* was detected by molecular methods in 1 out of 99 individuals analyzed in Salta⁸ and detected in 10 out of 218 individuals studied in Misiones⁴. In the latter province, different studies detected up to six species of *Entamoeba* infecting humans, mainly by morphological diagnosis^{4,23,29,42,43} (Fig. 1).

In light of scarce molecular data on *Entamoeba* species in Argentina, the present study aimed to: (i) determine the prevalence of *Entamoeba* species in human fecal samples from two populations of this country by microscopic methods, (ii) make a differential diagnosis by PCR and (iii) characterize the *Entamoeba* isolates at the SSU rRNA gene.

Material and methods

Study area and sample collection

Data for this study were obtained as part of a larger cross-sectional survey on intestinal parasitic infections and socioenvironmental conditions in human populations, which was conducted in schools, primary health care centers, and communal dining rooms located in two Argentine provinces: Buenos Aires and Misiones, between 2018 and 2019.

In Buenos Aires, 210 fecal samples were obtained from individuals aged 1–64 years residing in peripheral areas of the district of La Plata (34°55'17.22"S 57°57'16.31"W) and three municipalities in the South of Greater Buenos Aires (34°42'39.30"S 58°21'26.65"W; 34°42'33.45"S 58°18'17.22"W; 34°49'17.59"S 58°20'45.99"W). These individuals were characterized as living in poor socio-economic conditions, including no piped water connection, cesspit system, and informal works.

In Misiones, 283 fecal samples were obtained from individuals aged 1–64 years residing in metropolitan areas surrounding the locality of Aristóbulo del Valle (27°05'43"S 54°53'49"W). These individuals lived in houses made of sheet metal and wood, most of whom consumed water from wells and had no access to the sewage system.

After informed consent was obtained, each individual was provided with a sterile plastic vial containing 70% ethanol. Stool samples from 3, consecutive or intermittent days, were collected from each participant.

When participants were children, samples were collected by their parents or legal guardians.

Fecal samples were submitted to the Centro de Estudios Parasitológicos y de Vectores (CEPAVE-CONICET-UNLP, La Plata, Buenos Aires, Argentina). The samples were divided

into equal portions for microscopic examination and PCR sequencing analysis to differentiate all *Entamoeba* species.

Microscopic identification of *Entamoeba* cysts

Fecal samples were examined by light microscopy for the presence of *Entamoeba* and other intestinal parasites. The formalin–ethyl acetate concentration technique (FECT) and Willis flotation method were performed to detect the presence of parasites. First, each fecal sample, preserved in 70% ethanol, was homogenized with a ceramic pestle and mortar. The suspension was strained into a 15 ml conical tube through a double layer of gauze placed into a strainer or small funnel and centrifuged at 1500 rpm for 5 min. The supernatant was discarded. Then, 7 ml of formalin and 3 ml of ethyl acetate were added to the sediment, mixed, and centrifuged at 1500 rpm for 5 min⁴⁰. For Willis flotation, a saturated sodium chloride solution (S.G. = 1.2 g/ml) was used.

Molecular characterization of *Entamoeba* species in stool samples

DNA was extracted from fecal samples which were found to be positive for *Entamoeba* cysts. Before extraction, 500 mg of feces/ethanol suspension was centrifuged at 8000 g, and the pellet was washed three times with 1 ml of Dulbecco's phosphate-buffered saline (PBS). After centrifugation, the pellet was subjected to mechanical disruption by three freeze–thaw cycles. DNA was isolated using the ZR Fecal DNA MiniPrep™ Kit (Zymo Research, California, USA) following the manufacturer's instructions³².

Entamoeba-specific PCR

The DNA obtained was used for amplification of a 135-bp fragment harboring the SSU rRNA gene by PCR with the primers ED1 (5'-TACAAAGTGGCCAATTTATGTAAGTA-3'), EH1 (5'-GTACAAAATGGCCAATTCATTCAATG-3') for *E. dispar* and *E. histolytica* detection, respectively, with the unique reverse primer EHD2 (5'-ACTACCAACTGATTGATAGATCAG-3') following the cycling conditions reported by Gonin and Trudel¹⁵.

DNA from samples identified as *E. histolytica*–*E. dispar* complex by the microscopic diagnosis, but testing negative for the above PCR, were amplified using the primers Entam1 (5'-CACTATTGGAGCTGGAATTAC-3') and Entam2 (5'-GTTGATCCTAGTATTATATG-3')³⁹. Cycling conditions were: 5 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C, with a final extension at 72 °C for 2 min.

In both cases, PCR conditions were optimized in a final volume of 25 µl as follows: 1× GoTaq® buffer (Promega, Madison, USA), 0.2 µM dNTPs, 1 U/µl GoTaq® Hot Start polymerase (Promega, Madison, USA), 0.5 µM of each primer, 0.1 µg/µl BSA, 1.5 mM MgCl₂ and 4 µl of DNA as template. Positive DNA samples were obtained from *E. histolytica* and *E. dispar* human fecal samples provided by the Division of Parasitic Diseases and Malaria (Centers for Disease Control and Prevention).

PCR products were further purified and sequenced (Macrogen, Seoul, Korea), along with both primers used for each amplification, to validate positive PCR results and perform phylogenetic analyses. Forward and reverse sequences

obtained were assembled using the PREGAP and Gap4 programs of the Staden package³⁵. The consensus sequences were compared to the previously published sequences of the *Entamoeba* genus by using the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/blast). Sequences obtained were deposited at GenBank under accession numbers: MZ787759–MZ787761, MK541024–MK541027, OM985615–OM985620 and ON712729.

Phylogenetic analysis

The phylogenetic analysis was carried out using newly obtained *Entamoeba* sequences and matching them with other representative sequences. Furthermore, the available GenBank sequence of the *Endolimax nana* (accession no. AF149916) was used as an outgroup (Supplementary Table 2). DNA sequence data were aligned using the CLUSTALW program⁶, with default options for introducing gaps into the alignment. When needed, inconsistencies were checked and manually edited. Phylogenetic relationships were inferred by maximum likelihood (ML) using MEGA X software²² and the Bayesian inference (BI) as implemented in MrBayes Ver. 3.2.6 software³⁰. The best DNA substitution model (T92+G) was estimated using the jModelTest program²⁶. Nodal support of ML analysis was estimated by performing 1000 bootstrap replications. The BI analysis was performed using Markov chain Monte Carlo (MCMC) chains for 1 000 000 generations with sample frequency set at 100. The first 25% of the trees sampled were discarded as 'burn-in'. This number of generations was considered sufficient because the SD dropped below 0.01. A pairwise distance matrix among *Entamoeba* sequences was calculated using the nucleotide p-distance algorithm implemented in MegaX²².

Statistical analyses

The prevalence of parasitized individuals and parasitic species was calculated. A sample was considered positive when at least a parasitic species was detected by any morphological or molecular method. The prevalence was calculated as the number of parasitized individuals divided by the total number of individuals analyzed, expressed in terms of percentage.

The relationships among parasitism and infection with *Entamoeba* species and intestinal symptoms were analyzed statistically. The Chi-squared test (χ^2) was used to determine the independence between the variables. In all cases, a *p*-value of <.05 was taken to indicate significance. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated.

All statistical analyses were performed using R software version 4.2.1²⁸.

Ethics approval

This study was approved by the Bioethics Consultative Committee of the National University of La Plata (Exp. No. 100–20 120/18) and the Research Ethics Committee Hospital El Cruce (Exp. No. 2919/1799/18). This research was conducted without affecting the physical, psychological or

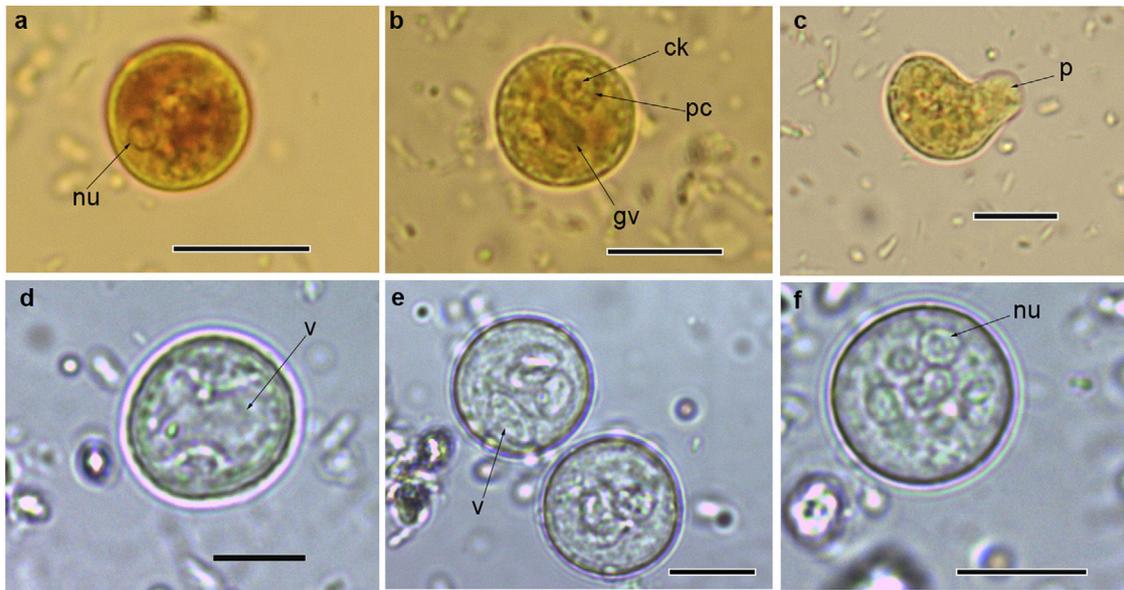


Figure 2 Cysts and trophozoites of *Entamoeba* found in populations from Argentina: (a and b) immature cysts of *Entamoeba* complex staining with Lugol; (c) trophozoite of *Entamoeba* complex staining with Lugol; (d and e) immature cysts of *E. coli*; (f) mature cyst of *E. coli*. Abbreviations: nu: nucleus; ck: central karyosome; gv: glycogen vacuole; pc: peripheral chromatin; v: vacuole; p: pseudopodia. Scale bars: a–f = 10 μ m.

moral integrity of the participants, and protecting their identity, complying with the ethical standards established by the Nüremberg Code (1947), the principles proclaimed in the Universal Declaration of Human Rights (1948), the Declaration of Helsinki (1964) and its successive amendments. Special attention was also paid to Article 5 of the Regulation Decree of National Law 25.326. In addition, all families involved were given the results of the parasitological diagnosis, and positive cases were referred to the nearest health center to receive the corresponding antiparasitic treatment.

Results

Entamoeba spp. prevalence

A total of 493 individuals aged 1–64 years were examined for *Entamoeba* cysts. The microscopic examination showed *Entamoeba* spp. in 12.4% (61/493) of the samples (Fig. 2). Among these, 86.9% (53/61) were identified as *E. coli* and 18.0% (11/61) as *Entamoeba* complex. By using PCR, *E. dispar* was recognized in 10 (2.0%), and *E. histolytica* in one (0.2%) of the total samples (Table 1) (Supplementary Fig. 1). The prevalence of these species and other intestinal parasites found in the analyzed provinces are shown in Table 1.

Entamoeba infection was more prevalent in the population from Buenos Aires (14.8%: 31/210), registering the first sequence data of *E. histolytica* in this province. In Misiones, the prevalence of *Entamoeba* spp. was 10.6% (30/283) (Table 1).

E. coli was the most prevalent species in both populations, followed by *E. dispar* (Table 1).

Statistically significant associations were observed between *E. coli*–*E. nana* ($\chi^2=9.42$; $p=0.01$; OR=3.40 [1.49–7.74]).

Molecular characterization of isolates and phylogenetic analysis

Eight PCR amplicons (467–631 bp) were successfully obtained from isolates of *E. coli* (GenBank Acc. Nos. MK541024, MZ787759–60, OM985616–17, OM985619–20, ON713469). The alignment of these sequences showed 99.3% identity with four polymorphic sites. Pairwise genetic distance ranged from 0 to 0.58.

Four sequences (491–591 bp) of *E. dispar* (MK541026, MZ787761, OM985615, OM985618) were 100% identical. In addition, one sequence of *E. histolytica* (133 bp) (ON712729) was obtained.

The phylogenetic reconstruction based on the ML and BI analyses showed the same topology. Three high-supported clades were formed, which correlated with species of *Entamoeba*-producing cysts with the same number of nuclei (Fig. 3). First, sequences of *E. histolytica*, *E. dispar*, *E. moshkovskii*, and *E. bangladeshi* isolated from humans clustered together (clade A). Sequences of *Entamoeba hartmanni* were placed as a sister lineage to clade A. All together formed a strongly supported monophyletic group including all species with four nuclei per mature cyst. The isolate of *E. polecki*, which forms cysts containing a single nucleus, appeared as a sister clade.

The other well-supported group included *E. coli* sequences (eight nuclei per cyst). Within this group, two clades were distinguished: B and C. Clade B included subtype II *E. coli* sequences and clade C included subtype I. Our newly obtained sequences of *E. coli* were part of the subtype I group.

The sequence obtained for *E. histolytica* (ON712729) could not be aligned because it had a lower coverage.

Table 1 Prevalence of *Entamoeba* infection based on microscopy and PCR assay of human fecal samples according to Argentinian locations. Prevalence was calculated according to the total number of individuals analyzed in each province: 210 in Buenos Aires and 283 in Misiones. The prevalence of other intestinal parasites was also included. At the end of the table, parasite richness was indicated for each province.

	Buenos Aires (n total = 210)		Misiones (n total = 283)		Total (n total = 493)	
	n	Prevalence (%)	n	Prevalence (%)	n	Prevalence (%)
Parasitized individuals?	94	44.8	116	41.0	210	42.6
<i>Entamoeba</i> spp.	31	14.8	30	10.6	61	12.4
<i>Entamoeba coli</i>	28	13.3	25	8.8	53	10.7
<i>Entamoeba dispar</i>	4	1.9	7	2.5	11	2.2
<i>Entamoeba histolytica</i>	1	0.2	0	0	1	0.2
<i>Endolimax nana</i>	25	11.9	9	3.2	34	6.9
<i>Enteromonas hominis</i>	10	4.8	9	3.2	19	3.8
<i>Iodamoeba bütschlii</i>	1	0.5	1	0.3	2	0.4
<i>Giardia lamblia</i>	43	20.5	27	9.5	70	14.2
<i>Blastocystis</i> sp.	41	19.5	49	17.3	90	18.2
<i>Hymenolepis nana</i>	0	0.0	1	0.3	1	0.2
<i>Ancylostomideos</i>	1	0.5	1	0.3	2	0.4
<i>Strongyloides stercoralis</i>	0	0.0	1	0.3	1	0.2
<i>Ascaris lumbricoides</i>	1	0.5	1	0.3	2	0.4
<i>Trichuris trichiura</i>	0	0.0	1	0.3	1	0.2
Richness		10		12		

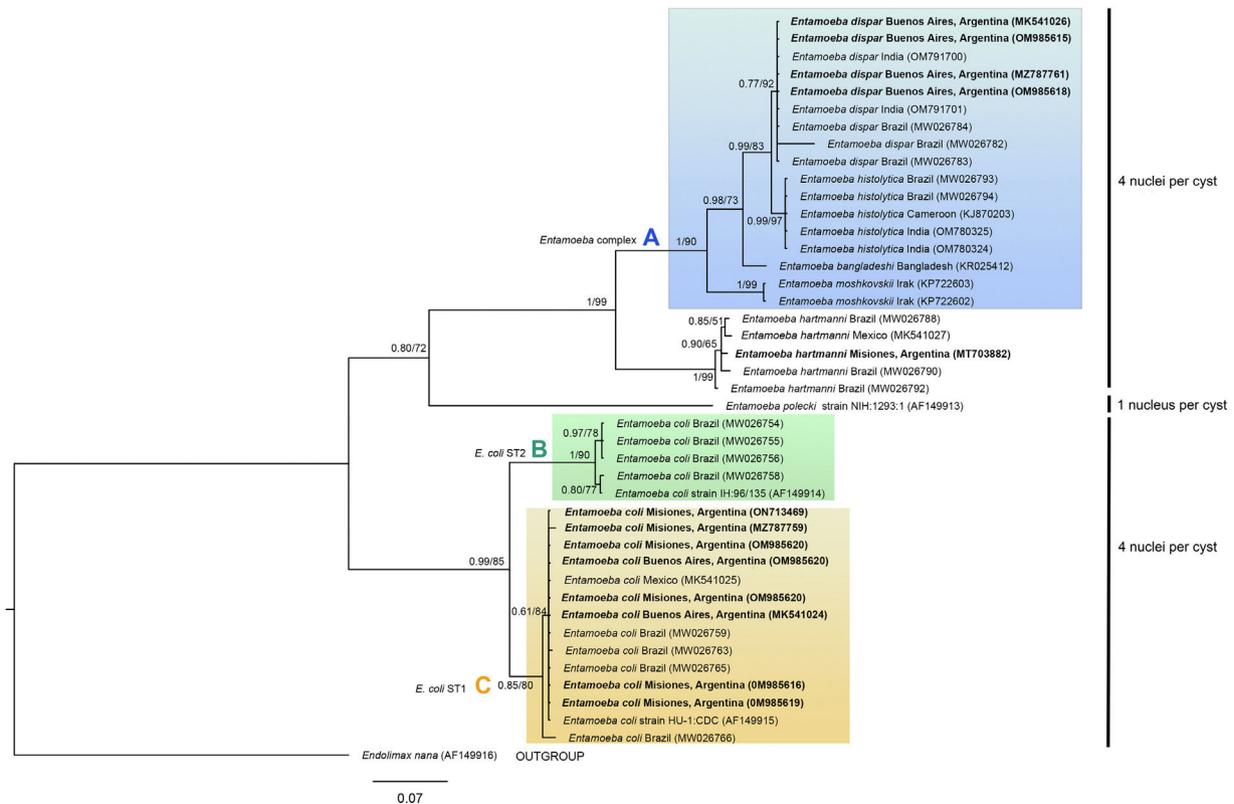


Figure 3 Phylogenetic tree constructed by the BI and ML methods based on 42 sequences of a partial region of the SSU rRNA gene under the substitution model HKY+G+I. Sequences are identified by taxon name and country of origin (when this data was available) and GenBank Acc. Nos. Sequences obtained in the present study are in bold. The posterior probabilities and the percentage of trees that clustered together based on the bootstrap test (1000 replicates) are shown next to the branch nodes.

Discussion

The molecular epidemiological information on *Entamoeba* species is still scarce in Argentina. This study provides new genetic data from *E. coli*, *E. dispar* and *E. histolytica*. The phylogenetic relationship inferred from SSU rRNA gene data was consistent with the classification of *Entamoeba* species based on the number of nuclei observed in the mature cysts.

In this study, the overall *Entamoeba* prevalence determined by morphological and molecular methods was 12.4%, being more prevalent in Buenos Aires than in Misiones (14.8% vs. 10.6%). In previous studies performed in Buenos Aires, the prevalence of *Entamoeba* spp. ranged from 8.9 (7/79) to 33.7% (27/80) and *E. coli* was the most prevalent species. In these studies, the diagnosis was made by conventional methods, such as formol-ethyl concentration and flotation methods, and detected *E. coli* as the most prevalent species (Supplementary Table 1). On the other hand, only one study detected *E. histolytica* in Buenos Aires by microscopic-based techniques (Ritchie's sedimentation, Willis's flotation, and trichrome staining)⁹. However, formalin-fixed samples were analyzed in this study, which means that the morphology of the trophozoites was not preserved. Therefore, it cannot be stated that the authors had correctly identified this protozoan.

In Misiones, the prevalence of *Entamoeba* spp. ranged from 70.6 (153/218) to 7.4% (36/483) and *E. coli* was also the most prevalent species (Supplementary Table 1). In addition, different studies revealed the presence of *E. histolytica*, *E. dispar* and *E. hartmanni* in native populations by molecular characterization of the SSU rRNA gene^{4,43}.

As described above, previous studies have reported a wide range of prevalence of *Entamoeba* infection in both provinces. Since this protozoan is primarily transmitted through the fecal-oral route²¹, the prevalence of the infection may be more closely related to socioeconomic factors and individual habits rather than to the edaphoclimatic characteristics of the provinces analyzed^{4,25}. In this study, the prevalence was slightly higher in Buenos Aires than in Misiones. While individuals in both provinces lived in impoverished conditions, the residents of Buenos Aires experienced a lack of access to piped water, while the majority of those living in Misiones relied on well water for consumption. In this sense, the source of drinking water has been identified as a significant risk factor for *Entamoeba* infections³. Therefore, these characteristics, rather than the environmental factors, may have strongly influenced the prevalence of this parasite infection in these areas.

Differential diagnosis of the pathogenic *E. histolytica* and nonpathogenic *Entamoeba* species is crucial for treatment decisions and public health knowledge¹⁵. In addition, the accurate diagnosis of non-pathogenic amebas is important since the infection with these protozoans indicates fecal exposure (e.g. by food or drinking water), and may suggest possible exposure to pathogenic organisms¹¹. In developing countries of the Americas, amebiasis is typically diagnosed by identifying parasite cysts or motile trophozoites by microscopic examination of the suspected fecal samples^{10,31}. The disadvantages of this conventional method include its low sensitivity and specificity, with false positive results due to the presence of other *Entamoeba* species¹⁰. As a consequence, several other diagnostic procedures, based on

parasite culture, serologic tests, antigen detection, and PCR, have been developed¹⁷. In this sense, PCR has been approved by the World Health Organization as the method of choice for the diagnosis of *E. histolytica*¹⁶.

In this work, a combination of microscopic analyses and PCR assays, with high sensitivity and specificity to detect *Entamoeba* species, developed by Gonin and Trudel¹⁵ and Verweij et al.³⁹ were performed. Thus, *E. coli* subtype I was detected in both Buenos Aires and Misiones populations, and a case of *E. histolytica* was detected in Buenos Aires. Regarding *E. coli*, it is a species complex characterized by the extensive diversity of cryptic sequences in the genes of the small subunit of ribosomal RNA, which has led to the recognition of two distinct subtypes (ST1 and ST2)³⁶. There are scarce molecular data on this species in South America. In fact, this is the first study to molecularly characterize isolates of this protozoan species from Argentina. *E. coli* ST1 and ST2 were found in Brazil, while ST2 was recorded in Perú and Ecuador³⁶. Stensvold et al.³⁷ have shown that ST2 was more common in samples collected outside Europe, whereas ST1 showed no geographical restriction. Similarly, this study provides the first molecular characterization of an *E. histolytica* isolate from Buenos Aires, which was obtained from a 24-year-old woman born in Paraguay but residing in Buenos Aires since 2015. Although the patient was asymptomatic, she received medical treatment and repeat parasitological testing was performed to confirm that she was parasite-free.

The same diagnostic approach was performed to diagnose *Entamoeba* species in a native community from Misiones⁴³. This methodology permitted the establishment of a more reliable diagnosis, detecting infections with *E. coli*, *E. dispar* and *E. hartmanni*⁴³.

The importance of an accurate diagnosis resides not only in treating the patients properly, but also in better understanding the epidemiology of amebiasis, which is a leading cause of severe diarrhea worldwide and accounts for 2.2 million disability-adjusted life years and around 55 500 annual deaths³⁸. Previous studies have shown that factors, such as the consumption of untreated contaminated water, sewage system, inadequate hygienic practices and social determinants were associated with a high prevalence of *E. histolytica* infections^{3,25}. As mentioned earlier, in this study, most of the individuals analyzed were characterized as living in poor socioeconomic conditions, without access to drinking water and a sewage network. In this sense, even though the prevalence of *E. histolytica* was low (0.2%), the overall prevalence of *Entamoeba* spp. infections (12.4%) may indicate the fecal contamination of water or food that people consumed.

Conclusions

Since the microscopic analyses allowed to screen all intestinal parasite infections and PCR was found to be a more reliable source of *E. histolytica* detection, a combination of these techniques was performed to study the prevalence of *Entamoeba* species in populations from Argentina. Among the *Entamoeba* complex species, *E. dispar* was the most prevalent. The first molecular record of *E. histolytica* and characterization of isolates of *E. coli* and *E. dispar* were supplied. Moreover, this study increases the information on the

distribution of these species in Argentina and the region of the Americas.

Author's contributions

AS conceived the study. AS participated in study design and molecular analyses, and analyzed and interpreted the data. AS performed the sampling, and parasitic examinations and wrote the draft of the manuscript. MLZ performed the sampling and parasitic examinations and contributed to discussing the results. GTN helped with parasitic examinations, coordinated the study and contributed to discussing the results. All authors read, revised and approved the final manuscript.

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Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ram.2023.05.003](https://doi.org/10.1016/j.ram.2023.05.003).

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