Last of the Human Protists: The Phylogeny and Genetic Diversity of *Iodamoeba*

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Associate editor: Andrew Roger

Abstract

Iodamoeba is the last genus of obligately parasitic human protist whose phylogenetic position is unknown. *Iodamoeba* small subunit ribosomal DNA sequences were obtained using samples from three host species, and phylogenetic analyses convincingly placed *Iodamoeba* as a sister taxon to *Endolimax*. This clade in turn branches among free-living amoeboflagellates of the genus *Mastigamoeba*. Two *Iodamoeba* ribosomal lineages (RL1 and RL2) were detected whose sequences differ by 31%, each of which is found in both human and nonhuman hosts.

Key words: *Iodamoeba*, protist, parasite, genetic diversity, phylogeny, evolution.

Iodamoeba is a genus of intestinal parasitic protist found in humans, nonhuman primates, and other animals. The genus was described by Dobell (1919), who also gave the name *Iodamoeba bütschlii* to the human parasite, and *Iodamoeba* from humans has been assigned to this species ever since. The name *Iodamoeba* derives from the conspicuous iodophilic glycogen mass present in *Iodamoeba* cysts (Supplementary fig. 1*A*, Supplementary Material online), often called a vacuole, although it is not membrane bound (Zaman 1972). Cysts are noticeably irregularly shaped, vary in diameter with a mean of approximately 10 μm (Dobell 1919; Taliaferro and Becker 1922), and usually have a single vesicular nucleus with a large spherical karyosome. Although mitochondrial structures were reported by Brown (1958) and Dutta (1962), ultrastructural studies did not confirm their presence (Zaman 1972). The life cycle comprises a trophozoite stage, found in the colon where it ingests bacteria and multiplies by binary fission (Dobell 1919; Rodenhuis 1919), and a cyst stage, responsible for transmission. Although originally placed in the family Entamoebidae together with *Entamoeba*, *Dientamoeba*, and *Endolimax* (Chatton 1925), to date, DNA sequence data have not been available for *Iodamoeba* and, therefore, its phylogenetic relationships remain unconfirmed. It is also not known whether humans and nonhumans are hosts for the same or different species. In this report, we finally answer most of the outstanding questions regarding this, the last genus of human parasitic protist to be investigated.

DNA was extracted from purified *Iodamoeba* cysts (Lebbad et al. 2008; Supplementary fig. 1*B*, Supplementary Material online), directly from feces, or from primary culture (table 1). Complete and partial *Iodamoeba* small subunit (SSU) ribosomal DNA (rDNA) sequences were obtained directly from polymerase chain reaction products or from clones thereof, using a wide range of primers (Supplementary table 1, Supplementary Material online).

Table 1. Samples Used and Sequences Produced for Phylogenetic Analyses.

DNA Sample ID	Source of DNA	Host	Geographical Info (Travel/ Origin)	Sequence ID	Sequence Length (bp)	GenBank Accession No.[c]	Sequence from PCR Products/Clone	*Iodamoeba* RL
EM081	Cysts	*Homo sapiens*	Thailand	EM081-6	1,752	JN635745	Clone	1
				EM081-3.1	1,961	JN635746	Clone	1
1074	Feces	*H. sapiens*	NA[b]	1074	2,376	JN635741	PCR	1
82	Feces	*H. sapiens*	NA	82	2,193	JN635742	PCR	1
28	Feces	*H. sapiens*	NA	28	509	JN635743	PCR	1
215	Feces	*Macaca fascicularis*	NA	215	150	NA	PCR	1
EM080	Cysts	*H. sapiens*	Cuba	EM080-I	2,215	JN635740	Clone	2
				EM080-A-H	252–257	JN635747–51	Clone	2
Mabel	Culture[a]	*Sus scrofa*	UK	Mabel	1,190	JN635744	Clone	2

NOTE.—PCR, polymerase chain reaction.

[a] Clark et al. (2006).

[b] NA, not available.

[c] GenBank accession number for sequence 215 is not available. The sequence can be downloaded from supplementary file 1 (Supplementary Material online).

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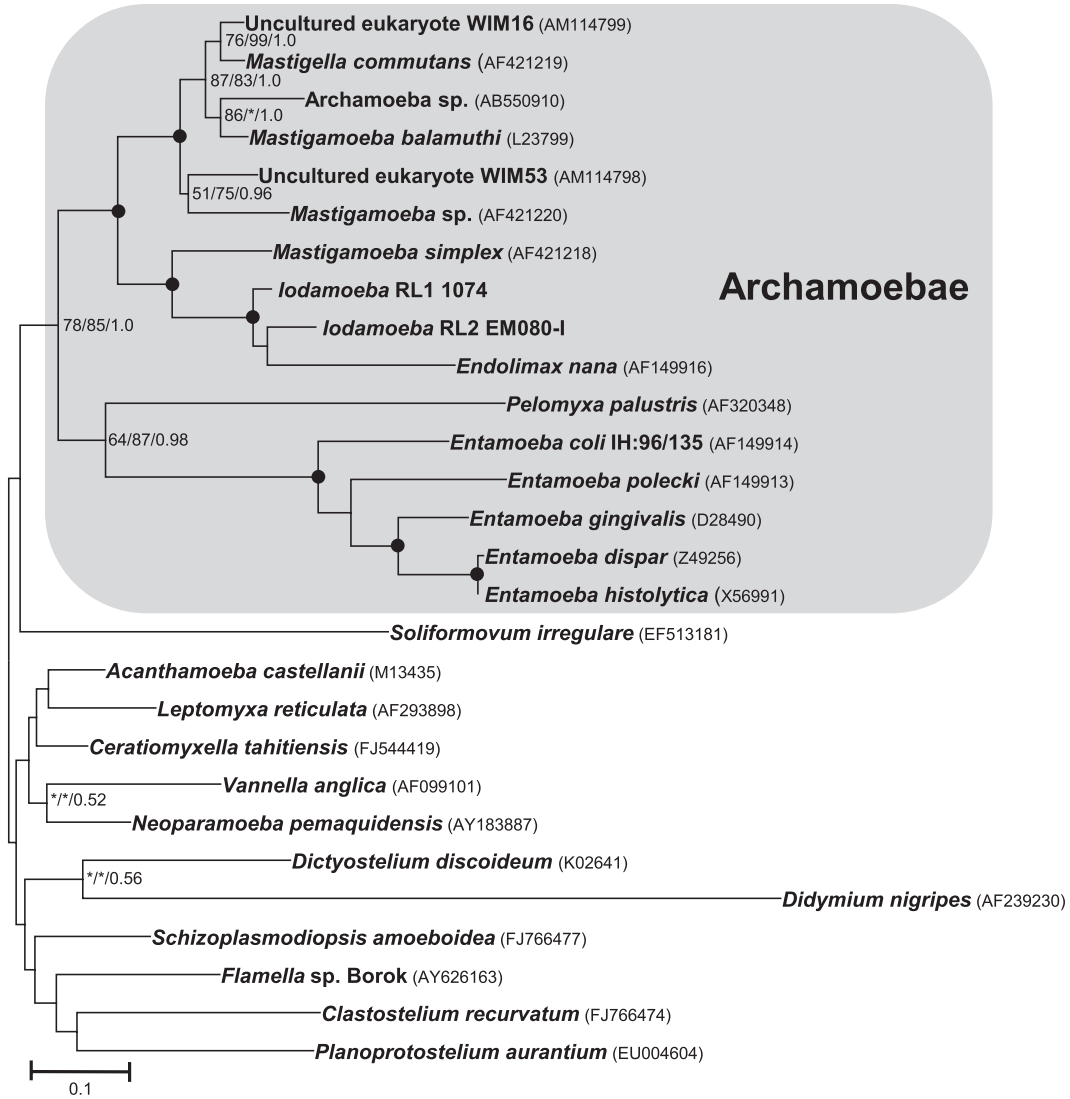

Fig. 1. Phylogenetic position of *Iodamoeba*. The analysis used 1,430 unambiguously aligned positions from 14 archamoebae, 2 *Iodamoeba*, and a broad selection of 12 non-archamoeba amoebozoan sequences. Alignments were generated using Molecular Evolutionary Genetics Analysis version 5 (MEGA 5) (Tamura et al. 2011) and the inbuilt Multiple Sequence Comparison by Log - Expectation alignment algorithm, then edited. Phylogenetic analyses used three different approaches: distance-based analysis (MEGA 5) used the Neighbor-Joining algorithm and the maximum composite likelihood model, whereas Bayesian (MrBayes 3.1.2; Huelsenbeck and Ronquist 2001) and maximum likelihood (ML; MEGA 5) analyses both used the general time-reversible model of nucleotide substitution with four categories of among-site rate variation and the proportion of invariant sites, selected as best using ModelTest (MEGA 5). Statistical support for distance and ML trees was evaluated using bootstrapping (1,000 replicates). The Bayesian analysis used four Markov chain Monte Carlo strands and 5,000,000 generations, with trees sampled every 100 generations. In the Bayesian analysis, the final average standard deviation of split frequencies was less than 0.01. A consensus tree was produced after excluding an initial burn-in of 25% of the samples, as recommended. The ML tree is shown. Bootstrap values and posterior probabilities from the three types of phylogenetic analyses are shown in the following order: ML/Distance/Bayesian. Nodes where both bootstrap values are >95% and the posterior probability is >0.95 are indicated by black circles. Bootstrap values of <50 or posterior probabilities of <0.50 are indicated by an asterisk, and where all three analyses show these low support values the node is not labeled.

Our results indicate a remarkable degree of genetic diversity within *Iodamoeba*. The sequences obtained fall into one of two ribosomal lineages (RLs) (table 1 and fig. 1) with a genetic divergence of 31%. Even within each RL, a substantial degree of diversity exists (Supplementary fig. 2, Supplementary Material online).

No two sequences from *Iodamoeba* DNA samples investigated in the study were identical. Substantial genetic diversity (8%) is seen among six clones from EM080 (table 1 and Supplementary fig. 3, Supplementary Material online), and the divergence between clones EM081-6 and EM081-3.1 in a 1,416-bp overlapping region is 6.7% (not shown). High levels of variation in the SSU-rDNA within strains is uncommon but has been reported previously in, for example, *Dientamoeba fragilis* (Silberman et al. 1996) and *Vannella simplex* (Nassonova et al. 2010). However, in this situation, we cannot differentiate between two possibilities: Each *Iodamoeba* cell may encode several distinct SSU-rDNA variants (intragenome variation) or most *Iodamoeba* infections are mixtures of multiple strains, each of which

has a single SSU-rDNA variant. Whatever the underlying basis of the variation, the remarkable levels of genetic diversity within single *Iodamoeba* infections have implications for the interpretation of boundaries between operational taxonomic units (OTUs). Caron et al. (2009) used a 95% identity level as their boundary between eukaryotic microbial OTUs. Our data indicate that *Iodamoeba* genes can exceed this 5% divergence value even within an individual infection.

Iodamoeba is well known from pigs and nonhuman primates; other examples of natural hosts include rodents, camels, and birds (Wenyon 1926; Kessel 1928; MacKinnon and Dibb 1938; Levine 1962; Ray and Banik 1964; Sano et al. 1980; Ponce Gordo et al. 2002; Howells et al. 2011). The fact that *Iodamoeba* sequence 215 from *Macaca fascicularis* is closely related to human RL1 sequences (data not shown), and that RL2 is found in both human and pig, suggests that existing *Iodamoeba* species names linked to specific hosts may not be valid. More data are needed to clarify the number and host range of RLs in *Iodamoeba* and, until such data are available, we suggest that the two lineages identified in the present study be referred to as *Iodamoeba* RL1 and RL2 rather than allocating species names to each, a similar approach to that recently suggested for novel lineages of *Entamoeba* (Stensvold et al. 2011).

In our phylogenetic analyses, *Iodamoeba*, *Endolimax*, and all mastigamoebids always cluster together to the exclusion of the remaining Amoebozoa with strong support, confirming the placement of *Iodamoeba* within this group (fig. 1). The respective lengths of the SSU-rDNAs of *Iodamoeba* and *Endolimax* are comparable (2.2–2.4 kbp) and in the range of typical mastigamoebid SSU-rDNAs, giving additional credence to the relationship. However, support for the well-established taxon Archamoebae as a whole is only moderate except in Bayesian analysis.

The sister taxon relationship of the two genera *Iodamoeba* and *Endolimax* is highly supported but, surprisingly, whereas monophyly of the two *Iodamoeba* sequences was supported by a high bootstrap value in distance-based analyses, statistical support in Bayesian and maximum likelihood analyses was absent. Manual comparison of the two *Iodamoeba* sequences with the *Endolimax* sequence revealed that shared SNPs were much more frequent between the two *Iodamoeba* RLs than were shared by *Endolimax* and either of the two *Iodamoeba* sequences.

In all our analyses, *Endolimax* and *Iodamoeba* share a specific common ancestor (fig. 1) and their branch emerges from within the free-living amoeboflagellate mastigamoebids rather than clustering with the parasitic *Entamoeba* spp. This indicates that adaptation to parasitism occurred independently at least twice in the Archamoebae, in the ancestor of *Entamoeba* and in the *Iodamoeba* + *Endolimax* branch; we cannot be sure whether the common ancestor of *Iodamoeba* and *Endolimax* was a parasite or not.

We set out to finally resolve the identity of the last genus of human parasitic protist to be studied at the molecular level—*Iodamoeba*. To fully resolve the phylogenetic position and taxonomic status of *Iodamoeba* and *Endolimax* based on SSU-rDNA, more data on intrageneric diversity for both *Endolimax* and *Iodamoeba*, but also *Mastigamoeba*, are needed. For now, we can conclude 1) that the genus *Iodamoeba* comprises at least two distinct RLs, both of which are found in humans and also occur in nonhuman hosts, 2) that substantial genetic variation is common in *Iodamoeba* from a single infection, 3) that *Iodamoeba* and *Endolimax* share a most recent common ancestor, and 4) that the genera *Iodamoeba* and *Endolimax* have arisen from within the mastigamoebids.

Supplementary Material

Supplementary figures 1–3, table 1, and file 1 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

We thank both Jaco Verweij and Egbert Tannich for providing DNA from *Iodamoeba*-positive samples. The work was done at Statens Serum Institut and London School of Hygiene and Tropical Medicine.

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