In 1996 and 1997, human-associated Cyclospora, a protozoan apicomplexan parasite, caused outbreaks of diarrheal disease in the United States and Canada that were associated with consumption of various types of fresh produce (1,2). Human-associated Cyclospora was previously referred to as “cyanobacteriumlike body” or “coccidialike body” and “big (or large) Cryptosporidium” (3). In 1993, Ortega et al. (3) proposed, on the basis of morphologic and sporulation characteristics, that this parasite be placed in the genus Cyclospora (Schneider, 1881) in the coccidian family Eimeriidae (Minchin, 1903). Although the species designation Cyclospora cayetanensis was given in 1994 to Peruvian isolates of human-associated Cyclospora (4), it is not yet known whether all human Cyclospora isolates belong to the same species.

Phylogenetic analyses by Relman et al., which included human-associated Cyclospora and three Eimeria species (two avian and one mammalian), supported the conclusion that Cyclospora and Eimeria belong to the same family of coccidian parasites (5). However, the authors noted that this apparent relatedness should be reevaluated when molecular data became available for additional Eimeria species and for Isospora belli, another coccidian parasite that causes diarrheal disease in humans.

Recently, the complete sequences of the small subunit ribosomal RNA (SSU-rRNA) gene of isolates of seven additional Eimeria species (six avian and one mammalian) were submitted to GenBank (6). We used these sequences, in addition to those previously available for human-associated Cyclospora and several Eimeria species (5), to reevaluate the phylogenetic relatedness of Cyclospora and Eimeria. Both of the previous phylogenetic analyses included sequences for E. tenella and E. mitis isolates; thus, we used two sequences for each of these species.

The structurally aligned sequences were retrieved from the Antwerp rRNA database (7); Mitchell L. Sogin kindly provided the alignment of the sequences used for the original molecular classification of Cyclospora (5). In addition to these two alignments, sequences were aligned with the ClustalW program (8). The aligned sequences were subjected to phylogenetic analysis with Dnapars, Neighbor, and Dnal programs from the PHYLIP package (9), using Cryptosporidium parvum (a coccidian parasite) or Oxytricha granulifera (a ciliate) as outgroups (all alignments are available from the authors upon request).

The topology of the phylogenetic tree obtained with all three methods was equivalent for all alignments. The maximum likelihood tree generated by the Dnal program from an alignment based on Sogin’s approach is shown in the Figure. The topology of this tree, which includes human-associated Cyclospora and 12 isolates of 10 Eimeria species, is similar to that of the trees reported previously for Cyclospora and three species of Eimeria (5) and for nine species of Eimeria (6). The Cyclospora branch on the tree is between the branches of the eight avian and two mammalian Eimeria species that have been evaluated to date.

The results of the phylogenetic analysis strongly suggest that Cyclospora should be considered a member of the genus Eimeria, which is particularly noteworthy, since no organism
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Currently classified as an Eimeria species is known to be pathogenic for humans. Eimeria is the largest genus of coccidial parasites and reportedly includes more than 1,500 named species (10). However, the current criteria (11) for naming "new" species of Eimeria (e.g., host specificity, morphologic characteristics of oocysts, duration of prepatent and patent periods, location of the infection in the host, and pathogenicity) are suboptimal, and the available data for some Eimeria species named in the past are incomplete. Thus, some Eimeria species may be synonymous, and some organisms thought to belong to the same species may not. The possibility even exists that human-associated Cyclospora is synonymous with a previously named Eimeria species. No molecular data are available for the type species of the Cyclospora genus or for the Cyclospora species that are not known to be human-associated. Reclassification, on the basis of phylogenetic analysis, of human-associated Cyclospora as an Eimeria species may stimulate productive research by suggesting possible animal reservoirs of human-associated Cyclospora (which may or may not infect other animals). In addition, animal models and cell culture systems that have been developed for Eimeria may prove useful for Cyclospora. However, it remains to be seen whether the biologic characteristics of Cyclospora are similar to those of the Eimeria species to which Cyclospora is closely related on the basis of phylogenetic criteria.

We also have preliminary data indicating that I. belli and human-associated Cyclospora do not belong to the same genus or family. I. belli oocysts (kindly provided by Alison Grant of Project RETRO-CI in Abidjan, Côte d'Ivoire) were gradient-purified, I. belli-specific DNA was extracted, and the SSU-rRNA gene was polymerase chain reaction-amplified and sequenced (Pieniazek et al., unpub. data). Sequence similarity searches of GenBank and preliminary phylogenetic analysis indicate that I. belli shares a more inclusive clade with members of the family Sarcocystidae than with the Eimeriidae (data not shown).

Molecular methods are arguably the best techniques available for studying the relatedness among organisms (11). To avoid confusion, reports of identification of Cyclospora (Eimeria) in animal hosts or in the environment should be supported by molecular data. Reports based on morphologic features alone (12-14) may suffer from poor resolution of features needed for classification of closely related organisms. To improve our understanding of the taxonomy of human-associated Cyclospora, molecular evaluation of isolates of additional Cyclospora and Eimeria species, especially other mammalian species, is needed.

Figure. Phylogenetic tree for small subunit ribosomal RNA (SSU-rRNA) sequences of Cyclospora (marked by an arrow) and 12 isolates of 10 Eimeria species. Maximum likelihood analysis results using Cryptosporidium parvum as an outgroup are shown (ln likelihood = -5,421.96594). After analysis, the outgroup branch was removed to improve the readability of the tree. GenBank accession numbers for the sequences: human-associated Cyclospora sp. – U40261, C. parvum – L16996, E. acervulina – U67115, E. bovis – U77084, E. brunetti – U67116, E. maxima – U67117, E. mitis 1 – U67118, E. mitis 2 – U40262, E. mivati – U76748, E. necatrix – U67119, E. nieschulzi – U40263, E. praecox – U67120, E. tenella 1 – U67121, E. tenella 2 – U40264. Scale bar indicates an evolutionary distance of 0.01 nucleotides per position in the sequence.
References

1. Herwaldt BL, Ackers M-L, and the Cyclospora Working Group. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. N Engl J Med 1997;336:1548-56.
2. Centers for Disease Control and Prevention. Update: outbreaks of cyclosporiasis—United States and Canada, 1997. MMWR Morb Mortal Wkly Rep 1997;46:521-3.
3. Ortega YR, Sterling CR, Gilman RH, Cama VA, Diaz F. Cyclospora species—a new protozoan pathogen of humans. N Engl J Med 1993;328:1308-12.
4. Ortega YR, Gilman RH, Sterling CR. A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. J Parasitol 1994;80:625-9.
5. Relman DA, Schmidt TM, Gajadhar A, Sogin M, Cross J, Yoder K, et al. Molecular phylogenetic analysis of Cyclospora, the human intestinal pathogen, suggests that it is closely related to Eimeria species. J Infect Dis 1996;173:440-5.
6. Barta JR, Martin DS, Liberator PA, Dashkevicz M, Anderson JW, Feighner SD, et al. Phylogenetic relationships among eight Eimeria species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences. J Parasitol 1997;83:262-71.
7. Van der Peer Y, Jansen J, De Rijk P, De Wachter R. Database on the structure of small ribosomal subunit RNA. Nucleic Acids Res 1997;24:111-6.
8. Thompson J D, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673-80.
9. Felsenstein J. PHYLIP—Phylogeny inference package. Cladistics 1989;5:164-6.
10. Levine ND. Phylum II. Apicomplexa Levine, 1970. In: Lee JJ, Hutner SH, Bovee EC, editors. An illustrated guide to the protozoa. Lawrence (KS): Society of Protozoologists; 1985. p. 322-74.
11. Sogin ML. Evolution of eukaryotic microorganisms and their small subunit ribosomal RNA. American Zoologist 1989;29:487-99.
12. Zerpa R, Uchima N, Huicho L. Cyclospora cayetanensis associated with watery diarrhoea in Peruvian patients. J Trop Med Hyg 1995;98:325-9.
13. García-López HL, Rodríguez-Tovar LE, Medina de la Garza CE. Identification of Cyclospora in poultry. Emerg Infect Dis 1996;2:356-7.
14. Smith HV, Paton CA, Girdwood RWA, Mtambo MMA. Cyclospora in non-human primates. Vet Rec 1996;138:528.