

Human parasite finds taxonomic home

SIR — The phylogenetic analysis of ribosomal RNAs from *Blastocystis hominis* provides another interesting example of how molecular data can elucidate the taxonomic affinity of an organism that has proved intractable by traditional techniques. *B. hominis*, an obligately anaerobic protist inhabiting the human intestine, has been a taxonomic enigma since its initial description more than 100 years ago¹. It has variously been (mis)classified as the cyst form of a flagellate, an amoeba, a yeast and a sporozoan, due to the presence of multiple morphological forms in infected individuals¹⁻³. No flagellated stage has ever been identified in *Blastocystis*: it possesses mitochondria with tubular cristae, and its most characteristic feature is a crescent cap of heterochromatin in the nucleus. Because electron microscope studies have failed to place *Blastocystis* in any particular phylogenetic group, we undertook a molecular systematic study of *B. hominis* and a *Blastocystis* isolate from the guinea pig.

We sequenced the complete *Blastocystis* small-subunit ribosomal (r)RNA genes and aligned them with the homologous coding regions from organisms represent-

ing all main eukaryotic lineages. The two *Blastocystis* isolates are most closely related to each other; high bootstrap values in parsimony and distance analyses unequivocally place *Blastocystis* within the stramenopiles (see figure).

Stramenopiles, together with green plants, red algae, animals, fungi and alveolates, separated nearly simultaneously approximately one billion years ago⁴. Molecular phylogenies define stramenopiles as a complex and heterogeneous evolutionary assemblage that includes unicellular and multicellular protists, with both heterotrophic and photosynthetic representatives^{5,6}. Brown algae (kelp), diatoms, slime nets and water moulds are just a few examples of the diverse organisms found in this group. All stramenopiles possess mitochondria with tubular cristae and, unlike all other eukaryotes, have tripartite tubular hairs either on their cell surface or, more commonly, on their long anterior flagellum.

Blastocystis is a very unusual stramenopile. Its mitochondria do possess tubular cristae, but, because *Blastocystis* is anaerobic, the metabolic functions of the mitochondria are unclear (the mitochondria lack many elements of the classical

energy-producing pathway)¹. *Blastocystis* also lacks flagella and flagellar hairs. This probably results from a secondary loss of these features, as its closest relatives, the Proteromonadidae and Bicosoecida, have flagella and tubular hairs.

Despite continuing controversy over details of the *Blastocystis* life-cycle, this organism shares certain life-history traits with its sister taxon, *Proteromonas*. Both organisms are gut endosymbionts of vertebrates and encyst to an environmentally resistant form that appears to allow transmission between hosts^{7,8}. A prevalent *Blastocystis* morphotype has a membrane-bound 'central body' in which progeny appear to form by schizogony⁷, reminiscent of the multiplicative cysts of *Proteromonas*⁸.

Molecular analyses may provide insights into the recognition of new *Blastocystis* species and their host range. The small-subunit rRNA genes from the two *Blastocystis* isolates differ by 6.4% (see figure). This evolutionary distance is comparable to that observed between disparate animal⁹, plant⁹, fungi¹⁰ and stramenopile¹¹ species. Additionally, immunological and DNA hybridization analyses^{12,13} have revealed variation among *B. hominis* isolates from humans, raising the possibility that more than one species can infect a host. This new information about the phylogenetic affinity of *Blastocystis* provides the first demonstration of a stramenopile that infects humans.

Jeffrey D. Silberman

Mitchell L. Sogin

Center for Molecular Evolution,
Marine Biological Laboratory,
Woods Hole, Massachusetts 02543, USA

Detlef D. Leipe

NCBI (GenBank), National Library
of Medicine,

National Institutes of Health, Building 38A,
8600 Rockville Pike,
Bethesda, Maryland 20984, USA

C. Graham Clark

Department of Medical Parasitology,
London School of Hygiene
and Tropical Medicine,
Keppel St, London WC1E 7HT, UK

Relationship of *Blastocystis* species among eukaryotes as determined by phylogenetic analyses of 16S-like rRNA gene sequences. *Blastocystis* (ATCC 50177 and 50578) 16S-like rRNA genes were amplified *in vitro* from genomic DNA, cloned, sequenced and aligned with homologous rRNA coding regions from other eukaryotes. Phylogenetic reconstructions using maximum-likelihood, Fitch-Margoliash, neighbour-joining and parsimony analyses show that *Blastocystis* is embedded within the stramenopile lineage (only values from 100 bootstrap replications of the latter three analyses supporting the major eukaryotic groups are shown). *Blastocystis* branches early in this lineage, as do the other heterotrophic stramenopiles. *Blastocystis* sp. ATCC 50578 was cultured from guinea pig faeces provided by C. Chrisp, Unit for Laboratory Animal Research, Ann Arbor. Sequences have been deposited with GenBank; accession numbers are: *B. hominis*, U51151; and *Blastocystis* sp., U51152. The aligned dataset is available on request.

