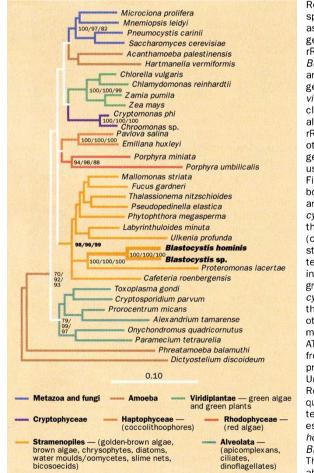
Human parasite finds taxonomic home

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 representing 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



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 eukarvotes, Phyloreconstructions genetic using maximum-likelihood, Fitch-Margoliash, neighbour-ioining 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 heterotrophic other stra menopiles. Blastocystis sp. ATCC 50578 was cultured guinea pig faeces from provided by C. Chrisp. Unit for Laboratory Animal Research, Ann Arbor. Sequences have been deposited with GenBank: accession numbers are: B. U51151; hominis. and Blastocystis sp., U51152. The aligned dataset is available on request.

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 membranebound 'central body' in which progeny appear to form by schizogony⁷, reminiscent of the multiplicative cysts of Proteromonas⁸

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.

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