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SOFTWARE TOOL ARTICLE

REVISED BlastoDB: first release of a community-driven multi-omics and epidemiological resource for *Blastocystis* biology and subtyping

[version 2; peer review: 2 approved]

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Abstract

BlastoDB (https://www.blastodb.com/) is developed as an open-access, community-driven resource dedicated to *Blastocystis*, one of the most common yet understudied intestinal protists. BlastoDB will offer the scientific community up-to-date, curated information on *Blastocystis* by integrating epidemiological data, microbiome profiles, multi-omics datasets (genomics, transcriptomics, proteomics, and metabolomics), reference sequences for subtypes, protocols, microscopy images, and related metadata. In this initial release, we describe the data model, database architecture, curation pipelines, and web interface, which together facilitate subtype classification, comparative and integrative analyses, and cross-study synthesis of epidemiological and experimental data. We outline submission and governance workflows designed to support community contributions, training activities, and sustainable curation under the "*Blastocystis* under One Health" COST Action (CA21105). Finally, we highlight planned extensions, including expanded metagenomic and metatranscriptomic content, automated genome quality assessments,

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metagenome-assembled genomes, and geospatial and analytical dashboards. BlastoDB provides a central, FAIR-aligned hub for *Blastocystis* data, images, and protocols, reducing technical barriers and fostering a collaborative ecosystem for studying this globally prevalent protist.

Keywords
Blastocystis; database; epidemiology; microbiome; multi-omics; subtypes; community resource; One Health

This article is included in the COST Actions gateway.

This article is included in the Blastocystis collection.

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REVISED **Amendments from Version 1**

This version of the article differs from the originally published version in three related respects, all arising from peer review. First, the description of the Subtype reference sequences and markers content category (Section 2.1) has been expanded to clarify that BlastoDB records, alongside each subtype assignment, the amplified region of the 18S rRNA gene, the primers used, and the sequencing strategy employed (e.g., Sanger sequencing or amplicon-based next-generation sequencing). This addition addresses the well-recognised problem of cross-study incomparability in *Blastocystis* subtyping caused by variation in these methodological parameters. Second, the description of the Microbiome and other multi-omics datasets category has been updated to specify that, for 16S rRNA-based microbiome data, the sequencing platform, the amplified 16S rRNA gene region, and the DNA extraction method are captured as mandatory metadata fields. Third, the list of core methodological descriptors in the Epidemiological and clinical metadata category has been extended to include the amplified gene region and primer set. No other substantive changes were made to the text, data, figures, or conclusions of the article.

Any further responses from the reviewers can be found at the end of the article

1. Introduction

Blastocystis is a single-celled microbial eukaryote and the most common intestinal protist in human faeces.[1] Although *Blastocystis* is frequently detected in epidemiological studies, its biology, epidemiology, and clinical significance remain incompletely understood.[2] The field is shaped by recurring issues: a lack of unified nomenclature, inconsistent diagnostic methods, uneven and inconsistent reporting of datasets and associated metadata. These factors significantly impede cross-study comparison and synthesis.[3]

Several platforms currently host *Blastocystis*-related data, including the PubMLST *Blastocystis* platform (https://pubmlst.org/organisms/blastocystis-spp), an 18S rRNA non-redundant reference set, NCBI, where a small number of reference genomes are deposited, and other sequence repositories.[3] However, these resources are typically centred on single marker genes or highly fragmented genome scale datasets. There are few well assembled and annotated genomes.[4–7] Regardless, almost none of the resources provide integrated multi-omics and epidemiological content with harmonised metadata (Table 1). As multi-omics datasets (genomic, transcriptomic, proteomic, metabolomic) integrating microbiome data[8,9] and clinical/epidemiological information accumulate,[10,11] there is a clear need for a curated, subtype-aware, and community-oriented resource that consolidates these datasets.

BlastoDB will be created as a Bioinformatic Resource Hub (BRH) by the "*Blastocystis* under One Health" COST Action, funded by the European Union's European Cooperation in Science and Technology (COST) programme.[12] The database is designed to enhance global understanding of *Blastocystis* within a One Health approach and to provide a single environment that can host and integrate different kinds of data on this often-overlooked gut protist and allow sharing with the scientific community. BlastoDB achieves this through the curation of subtype sequences, multi-omics datasets, culture and microscopic features, and by tackling several scientific challenges in *Blastocystis* research, including data source heterogeneity, lack of standardisation, underutilisation of available metadata, and fragmentated community.

Table 1. Current databases and links with *Blastocystis* data.

Database/link	Scope	Datatype	Strengths	Drawbacks	Reference
PubMLST – *Blastocystis* subtype/MLST database	Typing and allele/ST annotations	18S rRNA and MLST alleles, STs, isolate metadata	Gold standard for subtype classification	Focused mainly on marker gene	3
Zenodo 18S non-redundant database	18S rRNA reference dataset	Curated 18S rRNA Fasta	Ready-to-use reference for amplicon pipelines	Single locus only	13
Genoscope Genome Browser	Reference genome browser	Genome assemblies and annotations	Gene-level browser for reference genomes	Few genomes	14
NCBI (GenBank/ Genome/SRA)	General sequence repository	Genomes, raw reads, transcriptomes	Largest sequence database	Fragmented annotations, mostly non-curated	7

The objectives of BlastoDB are to consolidate subtype reference sequences and genomes, collate epidemiological and microbiome datasets, offer a simple interface for querying and visualising data, improve metadata standards, and encourage community contributions.[12] BlastoDB aims to harmonise different omics layers, including genomics, transcriptomics, proteomics, and metabolomics, and to connect these with epidemiological and microbiome information, protocols, and visual resources. The long-term goal is to support a comprehensive understanding of the role of *Blastocystis* in health and disease across hosts and environments.

In this manuscript, we introduce the first public release of BlastoDB. We detail its content, structure and curation, the main interface, and key use cases. We then consider how BlastoDB can develop into a comprehensive, sustainable reference for *Blastocystis* research.

2. BlastoDB, an integrated multi-omics and epidemiological resource

BlastoDB will be a user-friendly, open-access resource with simple, click-and-go navigation. The main utilities are accessible from the homepage by selecting the relevant sections (Figure 1). This first release focuses on making curated data and key tools available, while establishing the framework for future expansion.

2.1 Database content and structure

BlastoDB covers a broad set of data types and resources:

- **Subtype reference sequences and markers**

Curated *SSU* rRNA and other marker sequences with subtype assignments, including links to external schemes and reference sets where applicable. These form the backbone for subtype classification and phylogenetic analyses. For each subtype assignment, BlastoDB records the amplified region of the 18S rRNA gene, the primers used, and the sequencing strategy employed (e.g., Sanger sequencing or amplicon-based next-generation sequencing), enabling users to assess classification consistency and comparability across studies.

- **Genomes and transcriptomes**

Raw reads and assembled genomes and transcriptomes from cultured isolates, with annotations where available. These datasets will be linked to isolates, subtypes, hosts and studies.

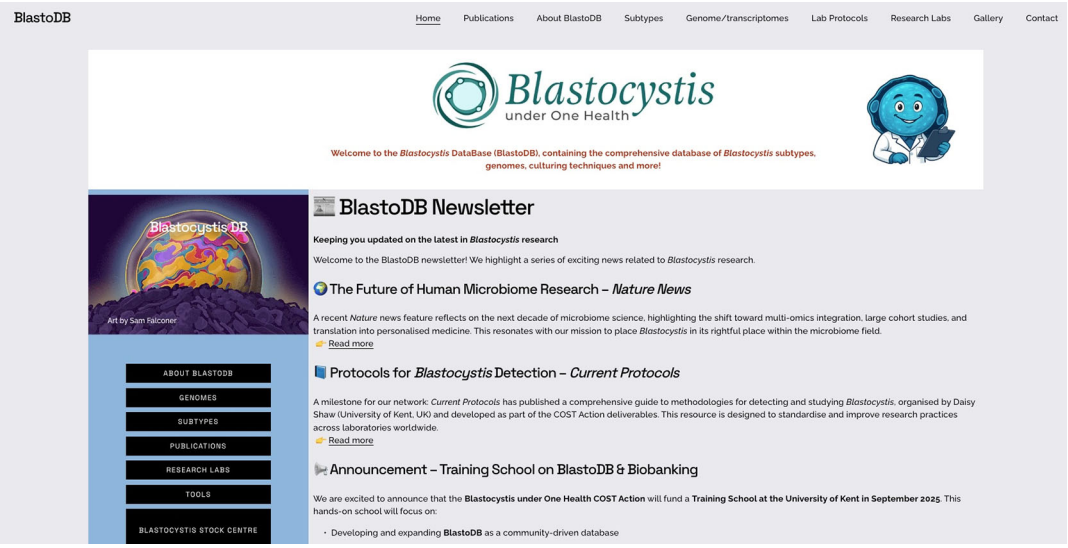


Figure 1. BlastoDB homepage. Screenshot of the BlastoDB homepage (https://www.blastodb.com/). The main navigation bar provides access to Subtypes, Genomes and transcriptomes, Data and protocols, and Labs and resources. A global search bar supports keyword queries across subtypes, hosts, locations and studies. Highlighted panels give quick access to featured datasets, recent updates and documentation.

- **Microbiome and other multi-omics datasets**

Culture-derived and host-associated datasets, including bacterial microbiome profiles, metabolomics and other omics outputs. Each dataset will be linked to subtype, host, sample type, culture conditions and any experimental (published) protocols. For 16S rRNA-based microbiome datasets, the sequencing platform (e.g., Illumina-based technologies), the amplified 16S rRNA gene region (e.g., V3–V4), and the DNA extraction method are captured as mandatory metadata fields, given their substantial influence on observed microbial community composition and cross-study comparability.

- **Epidemiological and clinical metadata**

Host taxonomy, geographic information (country, region), sampling context (community, hospital, cohort study), clinical phenotypes (e.g. symptomatic vs asymptomatic in the gut) and core methodological descriptors (sample type, extraction protocol, sequencing platform, amplified gene region, and primer set).

- **Protocols and laboratory resources**

Standard operating procedures for microscopy, molecular detection, culturing and subtyping, as well as links to *Blastocystis* culture collections and laboratories. This includes information on the laboratories on where the characterised isolates and specific subtypes can be obtained.

- **Images and visual resources**

Microscopy images from wet mounts and culture illustrating *Blastocystis* morphology will be linked with life cycle stages, subtypes, isolates, protocols (e.g. culture conditions) and datasets (Figure 2).

Metadata standards in BlastoDB include host taxonomy, geographic information, clinical phenotypes and methodological details. These will be stored in a database structure that connects isolates, sequences, experiments, protocols, laboratories, images and publications. The architecture combines this relational backend with a set of REST-style APIs and a browser-based frontend.



Figure 2. Example subtype page in BlastoDB. Representative subtype page illustrating the integration of marker sequences, genomes and metadata. The page summarises host range and geographic distribution for the subtype, lists curated marker sequences with download links, and displays available genomes and transcriptomes. Links to culture-derived multi-omics datasets, protocols and relevant laboratories are shown in dedicated sections.

2.2 Data acquisition and curation

BlastoDB will integrate data from three primary sources:

1. **Public repositories**

Genome assemblies, transcriptomes, marker sequences, and selected microbiome or metabolomic datasets are collected from GenBank/ENA/SRA, GEO, and related repositories using *Blastocystis*-specific queries and manual screening.

2. **Published supplements**

Detailed metadata, epidemiological information, and multi-omics measurements that are not readily accessible in public data archives are extracted from the supplementary materials of key *Blastocystis* studies. The minimum requirements for these depositories will be evaluated upon collection of all the data.

3. **Community contributions**

Researchers can directly submit new (raw or assembled) data to BlastoDB, including genomes, marker sequences, epidemiological datasets, microbiome profiles, culture observations, protocols, and images. Submission templates, validation rules, and documentation are available on the website. Where possible, submitters are encouraged to deposit primary data in long-term repositories (e.g., sequence archives, Zenodo) and link these to BlastoDB using accession numbers or DOIs.

Incoming data pass through a curation pipeline with the following stages:

- Ingestion and basic format validation;
- Mapping to the internal data model;
- Metadata normalisation and alignment to controlled vocabularies (e.g. host taxonomy, country codes);
- Subtype assignment and verification using curated marker reference sets and classifiers;
- Quality checks for genomes, transcriptomes and multi-omics data when relevant metrics are available; and
- Manual review of flagged records and ambiguous subtype calls.

Records will be given curation status labels (e.g. provisional, curated, deprecated). Curated entries are prioritised in searches, exports and subtype summaries.

Community contributions are encouraged through clear submission guidelines, DOI-aware workflows, and feedback mechanisms. Contributors are recognised in the database and in release notes.

2.3 Implementation and architecture

BlastoDB's backend is currently built as a relational database that stores key entities (isolates, sequences, omics datasets, epidemiological records, protocols, laboratories, images) and their interconnections. The database is accessed via a set of APIs that offer:

- Search and filter operations for isolates, subtypes, hosts, datasets and protocols;
- Programmatic retrieval of data in machine-readable formats (e.g. JSON, TSV, FASTA); and
- Endpoints for sequence classification and related utilities.

The web interface is built using a modern JavaScript framework. It consumes APIs and displays users with interactive tables, subtype pages, dataset summaries, and visualisation components. The system is deployed as containerised services behind a reverse proxy, which simplifies updates and scaling.

Each major content update will be treated as a release, with a release date, summary of changes and archived exports. This supports reproducible analyses and allows users to track changes in content and schema over time.

2.4 Functionalities, tools and visual resources

BlastoDB offers several tools and views to help users explore and use the data:

- **Homepage navigation**

The homepage provides direct entry points to Subtypes, Genomes and transcriptomes, Data and protocols, and Labs and resources, along with news on recent releases and featured datasets (Figure 1).

- **Search and filtering**

A global search bar supports keyword searches across subtype names, hosts, countries, studies and data types. Results can be filtered using facets such as subtype, host species, country, data type or year and exported as tables or FASTA files.

- **Subtype pages**

For each recognised subtype, BlastoDB provides a summary page with:

  - Host range and geographic distribution where known;
  - Curated marker sequences and download links;
  - Associated genomes, transcriptomes and other omics datasets;
  - Key epidemiological and clinical information;
  - Linked protocols and reference laboratories; and
  - Representative microscopy images and culture photographs.

- **Genome and transcriptome pages**

These pages will contain assembly metrics where available, isolate metadata (subtype, host, country, sampling context), links to external genome browsers or repositories, and cross-links to relevant subtype and dataset pages.

- **Sequence classifier and alignment tools**

Users can submit marker sequences (for example, *SSU* rRNA amplicons) to obtain subtype assignments with similarity scores and links to the closest reference sequences. Basic alignment and tree visualisation tools allow quick exploration of relationships among reference and query sequences.

- **Downloads and programmatic access**

Batch downloads of marker sets, genome collections or curated metadata can be triggered through the website. Programmatic users can access the same data via documented API endpoints.

- **Community and training sections**

Dedicated pages that list laboratories, culture collections, available protocols and training materials, as well as information on COST training schools and short-term scientific missions relevant to *Blastocystis.*

These tools together support tasks ranging from routine subtype assignment and dataset discovery to more elaborate meta-analyses and hypothesis generation.

3. Example use cases

This section illustrates how BlastoDB can be used in practice. The specific datasets and numbers can be adapted to real examples when you finalise the manuscript.

3.1 Subtype assignment and contextualisation of a new cohort

A research group performs *SSU* rRNA amplicon sequencing in a human cohort and wants to determine the distribution of *Blastocystis* subtypes and place their findings in context.

1. Representative *SSU* rRNA sequences are submitted to the BlastoDB sequence classifier.
2. The classifier returns subtype assignments with similarity scores and links to closest reference sequences.
3. Navigation to the subtype pages to review known host ranges and geographic distributions and to download curated reference FASTA files for pipeline validation.
4. Using the genomes and transcriptomes section, identifation of available genome resources for the dominant subtypes in the research group's cohort.
5. Export OF subtype- and host-specific metadata for comparative analyses and for planning follow-up work, such as culture attempts or targeted metabolomics.

BlastoDB therefore will provide a single environment for subtype assignment, reference selection and basic epidemiological contextualisation.

3.2 Comparing subtype distributions across hosts and regions

Another researcher is interested in how *Blastocystis* subtypes are distributed across human and animal hosts in different regions and take the following steps:

1. Search BlastoDB for all curated entries with subtype assignments and associated host and country metadata.
2. Filters are used to select the relevant host groups (for example humans, cattle, companion animals) and regions.
3. The filtered result table, including subtype, host, country, study and key methodological fields, is exported.
4. In an external statistical environment, subtype–host–region associations are analysed and differences in subtype prevalence between settings are tested for.

BlastoDB will streamline the process of discovering, harmonising and exporting data suitable for these analyses.

4. Discussion and future directions

In this initial release, BlastoDB tackles several longstanding bottlenecks in *Blastocystis* research. It consolidates curated subtype reference sequences, genomes, transcriptomes, proteomes, microbiome profiles, metabolomics, and other multi-omics datasets, along with epidemiological metadata, protocols, laboratory information, and visual resources. This integrated structure will enable researchers to explore more easily the connections between molecular diversity, epidemiology, microbiome context, and experimental biology.

By integrating standardised protocols, culture resources, and subtyping schemes within the same environment as the data, BlastoDB begins to address the methodological heterogeneity that has restricted cross-study comparability. It facilitates routine tasks such as subtype assignment and dataset discovery, while also lowering barriers to more advanced research, including strain-resolved comparative genomics, subtype-specific association studies, and combined analyses of host, microbiome, and parasite features. The inclusion of microscopy images and culture photographs adds an essential visual dimension, helping to link in silico and in vitro perspectives.

BlastoDB is, by design, a community-driven ecosystem rather than a static catalogue. Submissions of all types from researchers worldwide are welcomed, including new genomes, subtype sequences, epidemiological datasets, microbiome data, culture observations, protocols, images, and additional metadata annotations. Clear submission guidelines and DOI-aware workflows are intended to keep the resource aligned with FAIR principles while allowing it to expand in

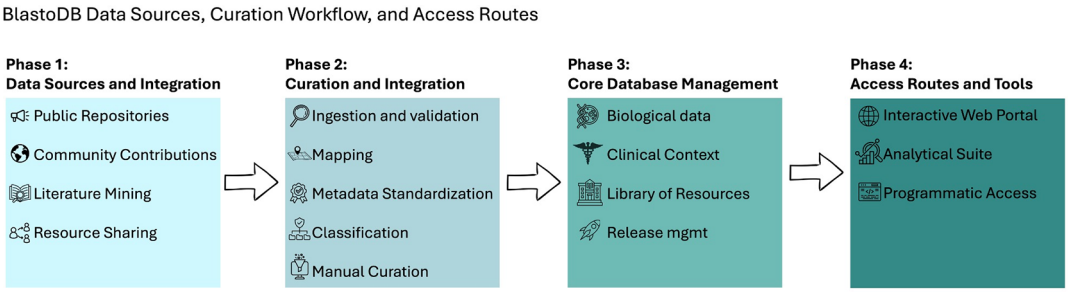


Figure 3. Schematic overview of the BlastoDB data flow and architecture. Data are collected from three main sources: public repositories (GenBank/ENA/SRA/GEO), published studies and supplementary materials, and direct community submissions. Incoming records pass through an ingestion and curation pipeline comprising basic validation, mapping to the internal data model, metadata standardisation, subtype assignment and quality control, and manual review. Curated entries are stored in the BlastoDB core database, which integrates isolates and subtypes, epidemiological and clinical metadata, microbiome and other multi-omics datasets, protocols, laboratory information and images. Versioned releases and documentation are generated from this core. Users access BlastoDB through a web interface (subtype and genome/transcriptome pages, data and protocol browser, image resources), built-in analysis tools (sequence classifier, alignment and tree viewers, filtered downloads) and programmatic access via APIs and release snapshots.

scope and geographic coverage (Figure 3). By integrating batch search tools, sequence classification, alignment visualisation, and basic phylogenetic exploration, BlastoDB reduces technical barriers for both novice and experienced users and enhances global capacity for *Blastocystis* research.

Sustainability remains a challenge. Long-term curation and infrastructure require stable funding and dedicated curator time. Embedding BlastoDB within the "*Blastocystis* under One Health" COST Action[12] and its working groups offers a strategic advantage by providing access to training activities, governance structures, and a large, engaged community. Future collaborations with microbiome consortia, pathogen databases, and generic data infrastructures can further enhance sustainability and interoperability. Over time, we aim to nurture an active "usage ecology" around BlastoDB by providing accessible training and outreach materials, including video-based tutorials and worked examples that illustrate typical workflows and best practices.

Looking ahead, there are several clear directions for development:

- Incorporation of metagenomic and metatranscriptomic datasets, including metagenome-assembled genomes and more detailed host and microbiome context;
- Automated genome quality assessment and improved tools for detecting contamination and misassembles;
- Geospatial dashboards for interactive visualisation of subtype distributions, hosts, and environmental contexts;
- Analytical modules for integrating multi-omics, microbiome, and clinical data, including machine-learning approaches to explore host–parasite interactions; and
- Richer, interactive comparative genomics environments that enable users to build and share in-browser analyses.
- AI-assisted curation and discovery tools that can harmonise and enrich metadata, flag inconsistencies, propose subtype assignments, and recommend relevant datasets, protocols and virtual biobank samples based on user queries and patterns in the data

Taken together, these additions will transform BlastoDB into a more dynamic platform. As data production speeds up across human, animal, and environmental interfaces, BlastoDB will offer a framework to integrate diverse datasets, facilitate reproducible research, and generate new insights into *Blastocystis* biology, its interactions with hosts, and its potential roles in health and disease.

Software availability

BlastoDB is provided as a hosted web application and is accessible via a standard web browser. At the time of publication there is no separate standalone software package or installable version of BlastoDB, and the underlying Squarespace-based implementation is not available as an independent open-source codebase. All functionality described in this article is accessible through the online platform. No additional software is required to use BlastoDB beyond a web browser and internet connection.

Ethics

Ethical approval and consent were not required for this article because it does not report any new studies involving human participants or animals. The work describes the design and implementation of a database resource that collates previously published and publicly available datasets.

Data availability

No new data are associated with this article. BlastoDB is an online resource that integrates previously published and publicly available datasets, which are cited in the main text and reference list. All underlying data remain available from their original repositories under the access conditions described in the cited publications.

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We thank members of the *Blastocystis* research community who shared data, protocols, images and feedback during the design and testing of BlastoDB. We are also grateful to the participants of COST Action training schools, workshops and short-term scientific missions, whose input has been essential for shaping both the resource and its future directions.

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

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The manuscript is approved.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical Microbiology and Parasitology (especially Blastocystis)

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 28 March 2026

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This manuscript presents a new valuable database for conducting reliable research on *Blastocystis*, beginning with properly collecting the samples and ending with the interpretation of molecular examination results. The focus on *setting up a* database *storing isolates, sequences, experiments, protocols, laboratories, images, and publications together* is extremely relevant, considering the current doubtful published epidemiological and molecular data of *Blastocystis* subtypes, their prevalence, and discoveries of the new ones. The authors present a new platform that aims both to collect reliable information on *Blastocystis* and to verify existing data, which will help avoid mistakes in identifying *Blastocystis* subtypes by researchers all over the world. It will be possible especially by using verified subtype reference sequences and genomes connected with epidemiological and microbiome information, protocols, and other resources.
The introduction of the manuscript highlights the difficulties in finding appropriate tools for analyzing genetic sequences from which *Blastocystis* subtypes can be confidently determined, which have become a challenge in recent years. The growing disinformation regarding the interpretation of molecular test results is increasingly irritating. Compared to the existing, similar tools, BlastoDB is the first curated, reliable, and comprehensive resource that combines multi-omics datasets (genomic, transcriptomic, proteomic, and metabolomic) accumulated for years by publishing the microbiome data as well as clinical/epidemiological information in one place. The authors mention a few websites/platforms where *Blastocystis* is one of many organisms described and the information about it is incomplete or fragmentary. According to that, almost none of the resources provide integrated content with high-quality metadata for comparison and successful reuse of data. BlastoDB provides a significant advance over previously published software.
The software is an open-access resource, freely available for users for non-commercial purposes, with simple navigation. It works as the authors described. BlastoDB offers several tools and views to help users explore and use the data. It adequately links the information necessary to investigate the genetic diversity of the protist and its epidemiology by the process of gathering, cleaning, and merging data from multiple, disparate sources into a single, centralized repository. It improves data quality, reduces redundancy, and enables efficient analysis by creating a "single source of truth." The authors of this article invite researchers to directly submit new data to the platform, making available on the website submission templates, validation rules, and documentation. The database content and structure are well described and presented on the figures as screenshots of the BlastoDB homepage and example subtype page, which is a helpful addition that makes it more accessible and clear. The authors describe in detail the purpose of the tabs on the website, how to use them, how to interpret the results obtained there, and how to use this data for further analysis. The section named “Example use cases” shows to the reader how the platform can be used in practice, which strengthens their understanding and demonstrates the software's functionality. The data’s primary sources were chosen very carefully and passed through a multi-stage curation pipeline. In addition, the selection process is clearly described and explained. The chosen documentation is sufficient to run the tool. The big advantage is that the authors state that there is no separate software package or installable version of BlastoDB, and all functions described are accessible online through a web browser and internet connection.
This manuscript introduces to the reader BlastoDB, a new software that can help develop methodologies for the detection and subtyping of *Blastocystis* so the research findings can be more reliable than the results published previously and additionally establishes a *Blastocystis* subtype and microbiome databank to make new research easier for all scientists as well as consolidate existing data. In general, the article is well written and structured. Each part of the manuscript presents the content clearly and orderly. The manuscript presents the assumptions of the created platform in an accessible and concise way, emphasizing the importance of collecting different types of data in one place.

Is the rationale for developing the new software tool clearly explained?
Yes

Is the description of the software tool technically sound?
Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?
Yes

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medical Microbiology and Parasitology (especially Blastocystis)

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 21 Apr 2026

Anastasios Tsaousis

We are grateful to the reviewer for her comprehensive and highly positive assessment of the manuscript. Her detailed reading of BlastoDB's rationale, design, curation pipeline, and use cases is greatly appreciated. We are pleased that she finds the platform to be a significant advance over previously available resources and that she considers the manuscript well written and clearly structured. She raises no specific concerns requiring changes to the manuscript. We note, however, that several of the strengths she highlights—particularly the importance of reliable metadata for subtype classification and the need for a curated, community-driven hub—are precisely the areas further strengthened by the revisions made in response to Reviewer 1's comments (see above). We believe these additions complement and reinforce the aims described in the manuscript. No changes to the manuscript were made in direct response to Reviewer 2's comments beyond those already described above.

Competing Interests: No competing interests were disclosed.

Reviewer Report 27 March 2026

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The article provides a detailed description of the structure and organization of the BlastoDB platform, as well as its various applications for integrating and standardizing information on one of the most prevalent intestinal protozoa worldwide. However, certain aspects can be strengthened to improve the clarity and reproducibility of the data presentation.
It is important to note that the assignment of subtypes (STs) in Blastocystis depends largely on the region of the 18S rRNA gene analyzed and the sequencing strategy employed.
In recent years, different regions of this gene (e.g., the “barcode” region vs. partial regions) and different methodological approaches (Sanger sequencing, amplicon-based NGS) have been used, leading to inconsistencies in classification and comparability across studies. For this reason, I suggest that information regarding the amplified region, the primers used, and the sequencing strategy be explicitly included alongside the subtype assignment.
Furthermore, the analysis of microbiomes associated with Blastocystis is a highly relevant component. In this context, it would be advisable for the sequencing platform used for microbiome analysis (e.g., Illumina-based technologies), as well as the amplified 16S rRNA gene region and DNA extraction methods, to be included alongside the reported data, given that these factors can introduce significant biases in the observed microbial composition and affect comparability between studies.

Is the rationale for developing the new software tool clearly explained?
Yes

Is the description of the software tool technically sound?
Yes

Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?
Partly

Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular parasitology. Molecular and microscopic diagnosis of intestinal parasites.

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard.

Author Response 21 Apr 2026

Anastasios Tsaousis

We thank the reviewer for her thorough and constructive feedback. Her comments identify important aspects of methodological transparency that are central to the mission of BlastoDB. We have addressed each point in full below, and the corresponding revisions are reflected in the tracked-changes version of the manuscript.

Comment 1.1: Subtype assignment and 18S rRNA gene region/sequencing strategy

Reviewer comment: "It is important to note that the assignment of subtypes (STs) in *Blastocystis* depends largely on the region of the 18S rRNA gene analyzed and the sequencing strategy employed. In recent years, different regions of this gene (e.g., the 'barcode' region vs. partial regions) and different methodological approaches (Sanger sequencing, amplicon-based NGS) have been used, leading to inconsistencies in classification and comparability across studies. For this reason, I suggest that information regarding the amplified region, the primers used, and the sequencing strategy be explicitly included alongside the subtype assignment."

Response: We fully agree with this important observation. Inconsistencies in the region of the 18S rRNA gene amplified and in the sequencing strategy used are well-recognised sources of subtype misclassification and cross-study incomparability. We have therefore revised the description of the **Subtype reference sequences and markers** content category in Section 2.1 to make explicit that BlastoDB records the amplified region of the 18S rRNA gene, the primers used, and the sequencing strategy (e.g., Sanger sequencing or amplicon-based NGS) alongside each subtype assignment. Specifically, the following sentence has been added: “For each subtype assignment, BlastoDB records the amplified region of the 18S rRNA gene, the primers used, and the sequencing strategy employed (e.g., Sanger sequencing or amplicon-based next-generation sequencing), enabling users to assess classification consistency and comparability across studies.” These fields are treated as mandatory metadata in the BlastoDB data model, consistent with the database’s broader goal of enabling rigorous cross-study synthesis.

Comment 1.2: Microbiome dataset metadata (sequencing platform, 16S rRNA region, DNA extraction) Reviewer comment: "The analysis of microbiomes associated with *Blastocystis* is a highly relevant component. In this context, it would be advisable for the sequencing platform used for microbiome analysis (e.g., Illumina-based technologies), as well as the amplified 16S rRNA gene region and DNA extraction methods, to be included alongside the reported data, given that these factors can introduce significant biases in the observed microbial composition and affect comparability between studies."

Response: We thank the reviewer for raising this point. It is well established that choice of sequencing platform, amplified 16S rRNA gene region (e.g., V3–V4), and DNA extraction method all substantially influence observed microbial community composition, and their omission from metadata makes cross-study comparisons unreliable. We have revised the

description of the **Microbiome and other multi-omics datasets** content category in Section 2.1 to specify that these three parameters are captured as mandatory metadata fields for 16S rRNA-based microbiome datasets. The following sentence has been added: “For 16S rRNA-based microbiome datasets, the sequencing platform (e.g., Illumina-based technologies), the amplified 16S rRNA gene region (e.g., V3–V4), and the DNA extraction method are captured as mandatory metadata fields, given their substantial influence on observed microbial community composition and cross-study comparability.” Furthermore, we have updated the description of the **Epidemiological and clinical metadata** category in the same section to expand the list of core methodological descriptors to include “amplified gene region” and “primer set” alongside sample type, extraction protocol, and sequencing platform. This ensures that these fields are consistently surfaced across all relevant metadata contexts within the database.

Competing Interests: No competing interests were disclosed.