scientific reports

OPEN

Construction of a preliminary digital parasite specimen database for parasitology education and research

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Despite advances in non-morphology-based parasite diagnostic techniques, traditional microscopy-based morphologic analysis remains essential for diagnosing parasitic infections. Therefore, parasite morphology is a crucial aspect of pre-graduate medical education. However, parasite specimen acquisition in developed countries is challenging because of the low rate of parasitic infections owing to improved sanitation. Hence, we acquired 50 slide specimens (parasite eggs, adults, and arthropods) from the Kyoto University and Kyoto Prefectural University of Medicine and created virtual slide data. All specimens ranging from parasitic eggs, adult worms, ticks and insects (typically observed under low magnification) to malarial parasites (typically observed under high magnification) were scanned successfully. These virtual slides were compiled into a digital database with folders organized by taxon. Explanatory notes in English and Japanese were attached to each specimen to facilitate learning. The data were uploaded to a shared server for institutions to facilitate practical training and research. The shared server enables approximately 100 individuals to access the data simultaneously. This database is expected to serve as an important resource for education and research in parasite morphology as additional parasitic slides and information are added in the future, contributing to the development of international parasitology education and future research.

Keywords Parasite, Virtual slide, Database, Parasitology, Pre-graduate medical education

The significant improvement in sanitary conditions in developed countries including Japan has significantly minimized the risk of parasitic infections[1,2]. Nevertheless, parasitic infections continue to be reported, as evidenced by the continued increase in the annual incidence of dysentery amebiasis[3,4]. This rise may be attributed to the globalization of infectious diseases and diversification of sexual behavior and food culture.

Detection of adult parasites and their eggs is essential for diagnosing parasitic infection. Hence, helping students gain an understanding of the characteristics of parasite morphology is an extremely important aspect of pre-graduate medical education programs. However, over the past two decades, training schools in Japan have been allocating significantly lesser time to parasitology education for medical technologists who play a central role in parasitology testing[5]. This trend is reflected globally in the decreasing number of hours that are devoted to parasitology lectures in medical student educational programs that include the treatment of parasitic diseases. Subsequently, this has led to concerns of decline in the ability of physicians to diagnose parasitic diseases in several countries[6–8]. A crucial factor that has contributed to this decline is the difficulty in obtaining specimens for educational purposes due to the reduced number of parasitic infections reported because of improved sanitary conditions. Consequently, only a limited number of parasite egg or body part specimens are available in these training schools. Furthermore, these specimens deteriorate over time owing to repeated use. Therefore, urgent measures need to be implemented to maintain the standard of parasitological education.

A recent global advance in the field of pathology is the development of the whole-slide imaging (WSI) technology for digitizing glass specimens[9,10]. WSI provides advantages such as prevention of specimen damage and deterioration, simplification of data storage and backup, improvement of search and browsing efficiency, and ease of specimen sharing over a wide area via the internet. Thus, WSI could improve the quality of education by providing an environment for students and researchers to efficiently advance their studies and research.

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Thus, the aim of this study was to develop a digital database utilizing existing slide specimens of parasite eggs, adult parasites, and arthropods to support international practical training and research, particularly within medical education programs.

Methods

The Kyoto University and Kyoto Prefectural University of Medicine provided 50 existing slide specimens of parasitic eggs, adult parasites, and arthropods for use in this study (Table 1). Some of the specimens were prepared at the university, whereas others were purchased from companies and museums. The slide samples did not contain any personal information and were intended for educational and research purposes only, including sharing.

Digital scanning of the slide specimens was performed by the Biopathology Institute Co., Ltd (Kunisaki City, Oita Prefecture, Japan). The SLIDEVIEW VS200 slide scanner by EVIDENT Corporation (Tokyo, Japan) was used to acquire the virtual slide data. Specimens with thicker smears were captured using the Z-stack function, which is a technique that varies the scan depth to accommodate thicker samples by accumulating layer-by-layer data[11]. Each slide specimen was digitally scanned individually. Slides with out-of-focus areas were rescanned as needed, and the clearest image was selected. The final images were then uploaded to a shared server (Windows Server 2022) provided by the institute to build a virtual slide database. All digital images were reviewed for focus and image clarity by authors before being incorporated into the database. The folder structure of the database was organized according to the taxonomic classification of the organisms.

Results

In total, 50 slide specimens of parasites (eggs and adults) and arthropods sections owned by Kyoto University and Kyoto Prefectural University of Medicine were included in this study (Table 1). All slide specimens typically observed using standard microscopy at low magnification (40x) such as parasite eggs, adults, fleas, and ticks, and at high magnification (1000x) such as malarial parasites were digitized (Fig. 1). The digitized data were uploaded to a shared server, and folders were created for each classification to store the specimen data (Fig. 2a). Additionally, each specimen was accompanied by a simple explanatory text to facilitate learning (Fig. 2b). Specimen names and descriptions were provided in English and Japanese to enhance accessibility and support use by domestic and international users.

Discussion

This database offers several advantages. First, virtual slides do not deteriorate over time, which facilitates storage for an extended time duration. Second, the data are widely accessible. The shared server enables approximately 100 individuals to access and observe the data simultaneously via a web browser on various devices such as laptops, tablets, or smartphones without requiring specialized viewing software. Third, confidentiality is ensured as access to the virtual slide database on the shared server requires the user to input an identification code and password, which is provided by the host organization. Consequently, this process necessitates the users to contact our organization to gain access to the database. The contacted user is allowed to use the database for educational and research purposes as previously agreed upon. Therefore, this methodology shows benefits such as the preservation of specimens of parasites that are becoming increasingly scarce in developed nations for applications in parasitological education and research.

Morphological diagnosis is essential for identifying parasitic infections; however, expertise in morphology is decreasing owing to the increasing use of non-morphological methods such as molecular biological techniques and antigen testing[12]. The use of nonmorphological tests has led to improved parasite detection and facilitated access to reliable diagnosis[13–16]. However, these tests typically target a limited range of known parasites; therefore, they may miss rare or emerging species and are hindered by inhibitory substances present in specimens[12]. In addition, specialized equipment and workflows required for these tests make them less accessible in resource-limited areas. Despite advancements in technology, microscopy-based morphologic analysis remains the gold standard for diagnosing many parasitic infections. The decline in morphological expertise has significant implications for patient care, public health, and epidemiology, highlighting the importance of preserving these traditional techniques[12]. The database in this study could potentially aid upcoming parasitologists and healthcare workers in acquiring valuable morphological knowledge.

The recent spurt in popularity of e-learning has led to its increased implementation in parasitological education[17,18]. Furthermore, the use of digital materials has reduced learning times[19]. Thus, in addition to being a valuable resource as teaching material for lectures and practical training in parasitology at various educational institutions for biology-related courses, this database doubles as a self-study material to compensate for shortened lecture time durations.

This study has several limitations. First, the specimens included in this study are restricted to the parasite and arthropod slides owned by Kyoto University and Kyoto Prefectural University of Medicine. There are plans to expand the database with additional national and international specimens in the future. Second, the digitization process depends on external services and equipment availability.

This database is available in Japanese and English, making it easy for non-Japanese-speaking users to utilize. Furthermore, information on parasites will be added to the database in the future, and it is expected to become a valuable resource contributing to education and research on international parasite morphology.

Major Group	Class	Name	Staining method	Sample location (Purchased from)
Protozoa		*Acanthamoeba* sp.	No staining	KUPM
		Cryptosporidium sp.	Kinyoun's acid-fast staining	KPUM (Meguro Parasitological Museum, Japan)
		Cryptosporidium sp.	Modified Fast acid staining	KPUM (Scientific Device Laboratory, Inc., USA)
		Cystoisospora belli	Modified Fast acid staining	KPUM (Scientific Device Laboratory, Inc., USA)
		Endolimax nana cyst・trophozoite	Trichrome staining	KPUM (Scientific Device Laboratory, Inc., USA)
		Entamoeba coli cyst・trophozoite	Trichrome staining	KPUM (Scientific Device Laboratory, Inc., USA)
		Entamoeba hartmanni	Trichrome staining	KPUM (Scientific Device Laboratory, Inc., USA)
		Entamoeba histolytica	Iron hematoxylin staining	KPUM
		Entamoeba histolytica, *Entamoeba coli* cyst・trophozoite	No staining	KPUM
		Giardia lamblia cyst・trophozoite	Trichrome staining	KPUM (Scientific Device Laboratory, Inc., USA)
		Giardia lamblia cyst	No staining	KPUM
		Plasmodium falciparum	Giemsa staining	KPUM
		Plasmodium falciparum	Giemsa staining	KU (Meguro Parasitological Museum, Japan)
		Plasmodium malariae	Giemsa staining	KPUM (Ward's science, USA)
		Plasmodium ovale	Giemsa staining	KPUM
		Plasmodium vivax	Giemsa staining	KPUM
		Toxoplasma gondii (tachyzoite)	Giemsa staining	KU (Meguro Parasitological Museum, Japan)
		Trichomonas vaginalis	trichrome staining	KPUM (Scientific Device Laboratory, Inc., USA)
Helminth	Cestode	*Dibothriocephalus nihonkaiensis* adult mature proglottid	Carmine staining	KPUM
		Dibothriocephalus nihonkaiensis eggs	No staining	KU (Kyoto Kagaku Co., Ltd., Japan)
		Dibothriocephalus nihonkaiensis eggs	No staining	KPUM
		Dipylidium caninum eggs	No staining	KU (Kyoto Kagaku Co., Ltd., Japan)
		Hymenolepis nana eggs	No staining	KPUM (TURTOX [formerly General Biological Supply House, Inc., USA])
		Spirometra mansoni (Whole body section)	H-E staining	KPUM
		Taenia saginata adult gravid proglottid	H-E staining	KU (Kyoto Kagaku Co., Ltd., Japan)
		Taenia saginata eggs	No staining	KU (Kyoto Kagaku Co., Ltd., Japan)
		Taenia saginata eggs	No staining	KPUM
		Taenia saginata eggs	No staining	KPUM (Wards [Ward's science], USA)
		Taenia solium eggs	No staining	KU (Kyoto Kagaku Co., Ltd., Japan)
	Trematoda	*Fasciola hepatica* eggs	No staining	KPUM (Wards [Ward's science], USA)
		Paragonimus sp. eggs	Unknown	KU (Kyoto Kagaku Co., Ltd., Japan)
		Paragonimus westermanii (lung section of dog)	H-E staining	KPUM
		Paragonimus westermanii eggs	No staining	KPUM (Ward's science, USA)
		Paragonimus westermanii eggs	No staining	KPUM
		Schistosoma haematobium eggs	No staining	KPUM (Wards [Ward's science], USA)
		Schistosoma japonicum adult	Derafield's hematoxylin staining	KPUM
		Schistosoma mansoni eggs	No staining	KPUM (Wards [Ward's science], USA)
	Nematode	*Ascaris lumbricoides* adult	Unknown	KU (Kyoto Kagaku Co., Ltd., Japan)
		Ascaris lumbricoides adult	No staining	KPUM
		Ascaris lumbricoides adult	No staining	KPUM
		Ascaris lumbricoides fertilized eggs	Gram staining	KU (Kyoto Kagaku Co., Ltd., Japan)
		Ascaris lumbricoides unfertilized eggs	No staining	KPUM
		Enterobius vermicularis adult	Unknown	KU (Kyoto Kagaku Co., Ltd., Japan)
		Enterobius vermicularis eggs	No staining	KU (Kyoto Kagaku Co., Ltd., Japan)
		Trichinella spiralis (cross section of mouse muscle)	H-E staining	KPUM
Arthropod		*Ctenocephalides felis*	No staining	KPUM
		Hemaphysalis longicornis larva	No staining	KPUM
		Pediculus humanus	No staining	KPUM
		Pthirus pubis	No staining	KPUM (TURTOX [formerly General Biological Supply House, Inc., USA])
Fungi		*Microsporidium* sp.	Trichrome staining	KPUM

Table 1. Samples used in this study. H-E, Hematoxylin Eosin; KPUM, Kyoto Prefectural University of Medicine; KU, Kyoto University.

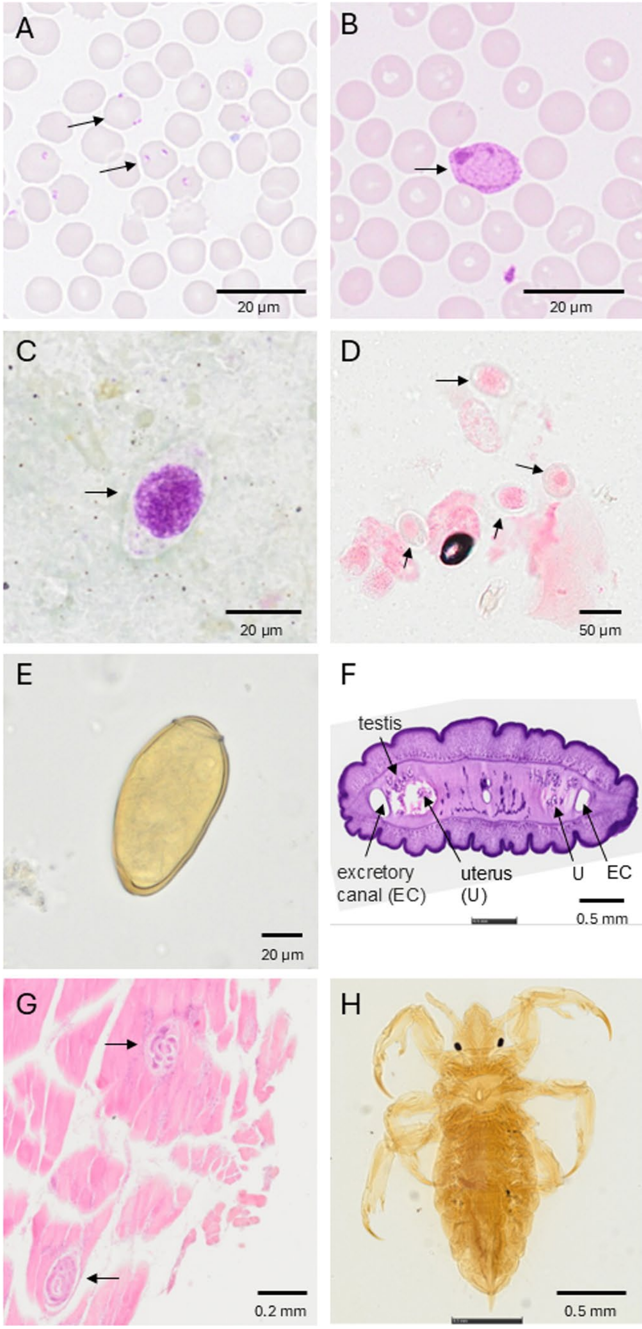

Fig. 1. Representative examples of acquired virtual slide data. (**A**) *Plasmodium falciparum* (Arrows): blood film showing multiple ring-form trophozoites stained with Giemsa. Notably, each of the two-ring form trophozoites in erythrocytic schizogony is small in diameter and recognized within a single red blood cell. (**B**) *Plasmodium vivax* (Arrow): blood film showing the macrogametocyte form. Notably, *P. vivax*-infected erythrocyte changes to a larger size than that of *P. falciparum*. The oval macrogametocyte stage in erythrocytic schizogony is recognized within a single red blood cell. The cytoplasm is blue, and the nucleus in the periphery is red after Giemsa staining. Many red and small granules, so-called Schüffner's dots, are recognized in the cytoplasm. (**C**) *Cystoisospora belli* immature oocyst (Arrow): an immature oocyst containing sporoblast is exhibited. Only the reddish sporoblast, except for the oocyst wall, is stained with Kinyoun acid-fast stain. (**D**) *Ascaris lumbricoides* fertilized eggs (Arrows); notably, many round eggs are stained with gram stain. Egg shells are not stained. (**E**) *Paragonimus westermanii* egg (Arrow): unstained fresh material. Notably, operculum (small cap of egg) is recognized. (**F**) *Taenia saginata* adult gravid proglottid (cross section): the void on either side is the excretory canal, the inner section on either side is the uterus, and the follicular testis is observed to surround them. Hematoxylin Eosin staining. (**G**) *Trichinella spiralis* larvae in mouse muscle: two arrows show nematode larvae sections. Hematoxylin Eosin staining. (**H**) *Pediculus humanus* male adult: giant claws in the latter legs and a pseudo penis in the tail region are apparent. No staining.

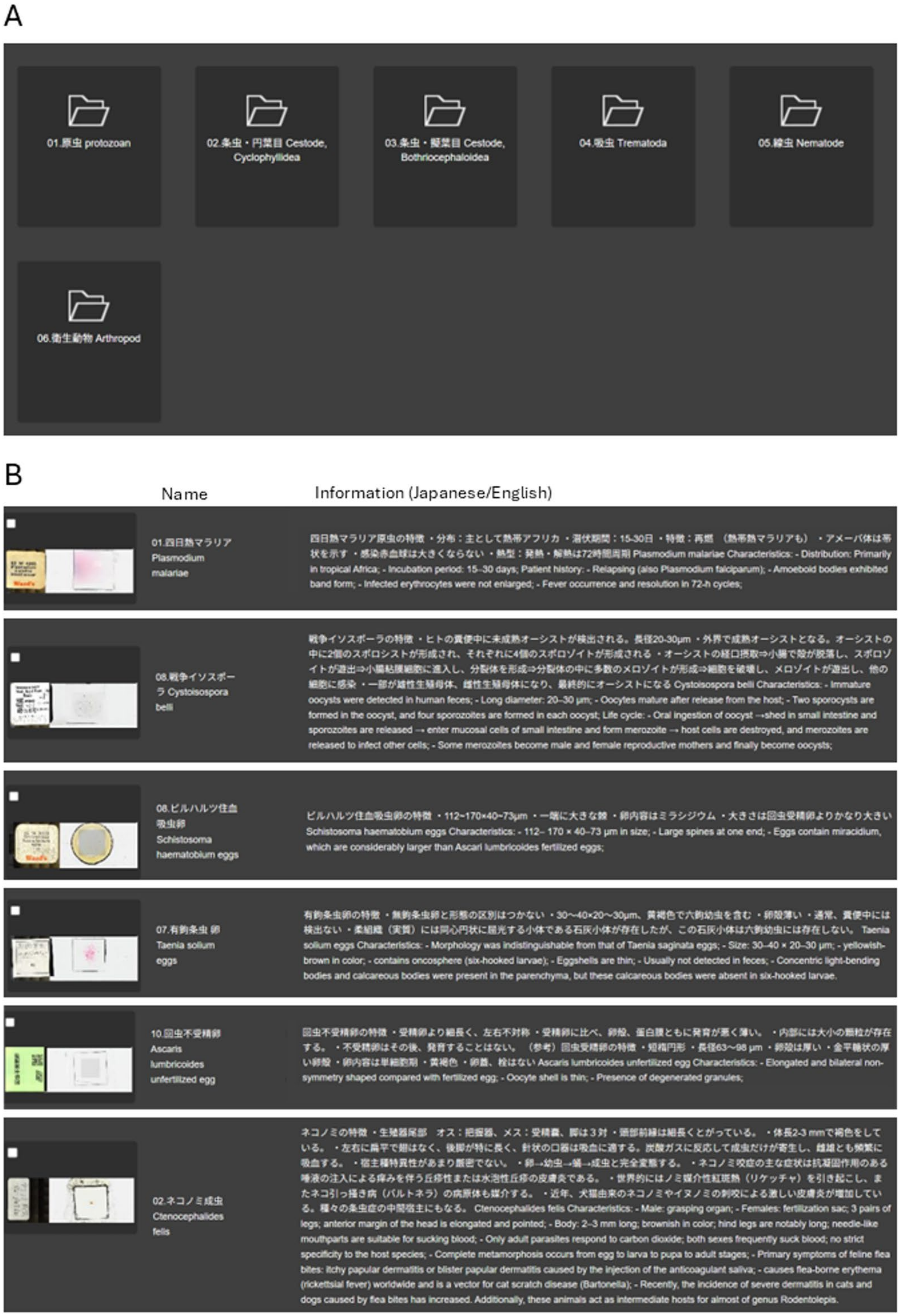

Fig. 2. Data in the virtual slide digital database. (**A**) Screen that appears after logging in. Folders were created for each class of parasite. Clicking on a folder will open a screen displaying a list of specimens such as B. To access the virtual slide database on the shared server, users must enter the identification code and password provided by the host organization. Therefore, users must contact our organization to obtain access. (**B**) Brief descriptions (in Japanese and English) of representative parasite specimen data. Click on a slide photo to open a virtual image of the slide in a separate window.

Data availability
All data generated or analyzed during the current study are included in this article. Further inquiries can be directed to the corresponding author.

Received: 29 March 2025; Accepted: 17 June 2025
Published online: 01 July 2025

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Acknowledgements
The authors thank Editage (www.editage.com) for the English language editing. This study was supported by the Japan National University Health Sciences Council's " Trial Project to Support the Revitalization of Education and Research in National University Health Sciences."

Author contributions
T.K. conceptualized the research. T.K., M.Y., and K.I. designed the study. T.K. drafted the manuscript. T.K., M.Y., K.I., and T.T. contributed to data acquisition and reviewed and edited the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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