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Multi-Class Microscopic Image Analysis of Protozoan Parasites Using Convolutional Neural Network

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Abstract: Protozoan parasites cause a wide range of devastating diseases in various kinds of organisms, including humans. It may be lethal if untreated promptly. To detect specific disease-causing parasites, a wide range of immunological and molecular technologies are now widely available. However, all of this depends on the worker's expertise and are time-consuming, error-prone, and expensive. With the development of technology, compared to traditional biological techniques, convolutional neural networks have reached excellent achievements in image classification, cutting costs while attaining an overall higher accuracy and eliminating human error. Many models include numerous convolutional layers and offer an accuracy between 90 and 95 percent. In this study, 4740 microscopic images of protozoan parasites from six classes with a balanced dataset and an 80–20% split were classified using three convolutional layers with stochastic gradient descent as an optimizer. A 5-fold cross-validation approach is used to evaluate the proposed method. We also examine and evaluate with deep learning models namely VGG16, ResNet50, and InceptionV3. The performance evaluation of the proposed model shows an accuracy of 94% with a precision range (of 0.83-0.99) and a recall range (of 0.76-1.00), respectively. The retrained model was able to recognize and classify all 6 different parasites. Except for class *Leishmania*, where 24% of images are incorrectly classified as *Plasmodium* and *Trichomonas*, the model demonstrates that most cases are correctly identified.

Keywords: Protozoan parasites, Convolutional neural networks, Multi-class classification, Microscopic images, Image analysis
Categories: I.2, I.4, J.3, L.3
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1 Introduction

Microorganisms are omnipresent in the environment, and they play a pivotal role in various natural processes while also causing several infectious diseases in all living

things worldwide. Certain protozoan parasites severely infect humans, aquatic animals, as well as wild and domestic animals, and generate broad clinical manifestations. Moreover, they can be found in almost every possible habitat. Incredibly, according to earlier reports, *Malaria is caused by Plasmodium*, similarly, Babesiosis (*Babesia*), sleeping sickness (*Trypanosoma),* Leishmaniasis (*Leishmania),* Toxoplasmosis (*Toxoplasma) and* Trichomoniasis (*Trichomonas)* causes severe infection in all living organisms [Akoolo et al., 2022]. According to the World Health Organization (WHO), the disease brought on by these parasites results in more than 20,000 deaths annually [Andrews et al., 2014, World Malaria Report 2019, Zhang et al., 2022]. Protozoan parasite identification requires various techniques because of the variations and uncertainties in the shape, density, and staining color of the parasites, the pathogenicity of particular species, and regional ranges. Therefore, a lack of precise identifications has restricted the knowledge of host-parasite specificity and susceptibility. The traditional methods of classifying and identifying microorganisms often rely on various laboratory techniques, including microscopic examination of blood or tissue samples, biochemical techniques like serological testing, and molecular techniques like polymerase chain reaction [Walochnik and Aspock 2012, Ajay et al., 2018]. Microorganism identification is a helpful diagnostic procedure for infected patients and animals. However, these traditional procedures are time-consuming, laborious, and error-prone and require a call for laboratory personnel's expertise and working experience. Therefore, an autonomous approach to parasite recognition is necessary to shorten the analysis process and improve diagnostic procedure accuracy. Image processing of largely ignored parasites including *Plasmodium*, *Babesia*, *Trypanosoma*, *and Leishmania* derived from samples has recently gained increasing interest with the application of deep learning techniques. In all cases, deep learning shows better accuracy and is substantially better than alternative strategies that are based on conventional medical imaging procedures [Hu et al., 2022, Jameela et al., 2022]. Among these, convolutional neural networks (CNN) and their variants have lately emerged as one of the most effective methodologies of choice for biomedical image analysis, and have already achieved remarkable results in the classification of microscopic images [Zhang et al., 2022], and medical image analysis [Hang et al., 2021], and other areas.

2 Literature Review

Previously published studies computerized the different image classifications. For example, LeNet CNN architecture was used to compare the quality of cell image data between a standard-resolution dataset and a high-resolution dataset using the microscopic image of three genera of bacteria and one yeast, achieving an accuracy of 80% [Treebupachatsakul and Poomrittigul, 2020]. In a similar study, a deep CNN was used on microscopic images of 5 different bacteria species, and the accuracy was 95% [Wahid et al., 2018]. Another study used blood smear malaria test data to examine the computational and predictive performance of four candidate deep-learning models that can be used for quick malaria case identification. It is discovered that basic convolutional neural network (B-CNN) and MobileNetV2 outperform VGG-19 fine-tuned and quantized models in terms of malaria detection performance, memory use, and inference time [Eze et al., 2021]. In another study, a fully connected CNN (U-Net)

was used to segment and classify different *leishmania* parasites, such as promastigotes, amastigotes, and adhered parasites [Górriz et al. 2018]. The Resnet18 model was trained using the pre-processed dataset, which mostly involved image cropping and the application of a thresholding method derived from microscope video of unstained thick blood smears from a mouse infected with *Trypanosoma brucei* [Jung et al., 2021]. In a different study, the cycle generative adversarial network (Cycle GAN) was used with the fuzzy C-means cluster algorithm to detect connections between microscopic and macroscopic related images of *Toxoplasma gondii*, and the accuracy for 400x and 1000x was 93.1% and 94%. respectively [Li et al., 2020]. TVNet, was used to automatically segment 3,158 microscopic images of *Trichomonas* with various appearances in diverse backgrounds. Extensive experiments demonstrate that TVNet model achieves superior segmentation performance and outperforms various cutting-edge object detection models both quantitatively and qualitatively [Li et al., 2022].

To the best of our knowledge, no studies have previously used deep learning to classify microscopic images of multi-class protozoan parasites. Using CNN with three convolutional layers, this study categorized 4740 microscopic images of six different protozoan parasites. The results demonstrate that the model can successfully identify multi-class protozoan parasites with an accuracy of 94%, and the results are consistent with earlier studies.

3 Material and Methods

3.1 Data Source

The microscopic images of various protozoan parasites (Figure 1) that are available at https://data.mendeley.com/datasets/38jtn4nzs6/3 have been retrieved via the publicly accessible Mendeley data repository [Li and Zhang, 2020]. The collection comprises 34,298 microscopic images of six groups of parasites and host cells, the count of microscopic images of the six types of parasites, and the number of images that were used in this study are shown in Table 1.

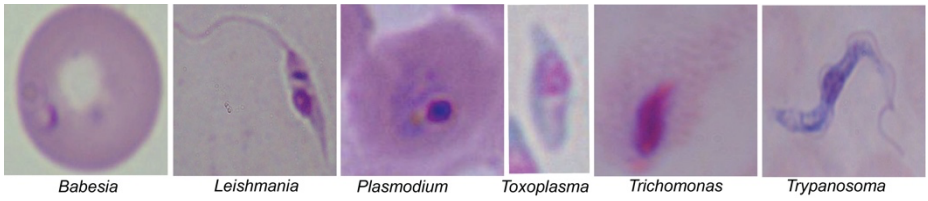

Figure 1: Microscopic images of various protozoan parasites Babesia, Leishmania, Plasmodium, Toxoplasma, Trichomonas, and Trypanosoma

Multiple parasites (6 classes)	Number of images	Number of images used in this study
Plasmodium 843	843	790
Babesia 1173	1173	790
Trypanosoma 2385	2385	790
Leishmania 2701	2701	790
Toxoplasma (1000x) 2933	2933	790
Trichomonas (1000x) 10134	10134	790

Table 1: The characteristics of the dataset used in this study

3.2 Train and Test Datasets

Table 1 (the class imbalance dataset) displays the unequal distribution of the number of microscopic images of various parasites across the six classes (number of images). Due to class imbalance, the conventional classifier favors the majority class or the class with the most instances. Thus a datasets with class imbalance need special attention [Sun et al., 2009]. The performance of the classifier can be improved by an ensemble of classifiers. However, ensembles are static and cannot be applied to imbalanced datasets [Cruz et al., 2018]. Additionally, based on experimental findings, it is known that the balanced dataset performs better than the imbalanced dataset [Potharajua et al., 2018]. Given the aforementioned words, the balanced multi-class classification dataset (790 images per class, Table 1) has been manually chosen and no data pre-processing applied to the images. As a result, out of 4740 microscopic images of various parasites from six classes used in this study, 3792 images were used as the training dataset (632 images for each class), and 948 images were used as the test dataset (158 images for each class), making up the split of 80–20% multi-class dataset (Figure 2).

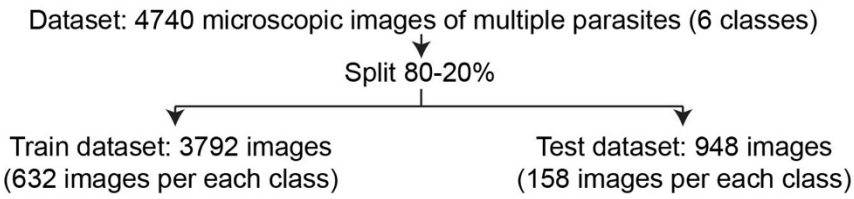

Figure 2: Dataset percentage split of 80–20%. 4740 microscopic images of multiple parasites belonging to six classes were split into 3792 images (train dataset) and 948 images (test dataset)

3.3 Proposed Convolutional Neural Network (CNN) Architecture

In this study, a Keras API (version 2.12.0), built on top of the TensorFlow platform (version 2.12.0) and uses Python programming, is employed as an analytical tool for multi-class protozoan parasites classification. The simplified CNN used in this study has three convolutional layers, the first convolutional layer uses 32 filters, the second convolutional layer uses 64 filters, and the third convolutional layer uses 128 filters.

Additional parameters include the 3×3 kernel size, one-pixel stride, HeUniform kernel initializer, the same padding, and the Rectified Linear Unit (ReLU) activation function. Following convolutional layers, there is a default max-pooling layer (i.e., 2x2 pool size), a flattened layer, a fully connected dense layer (128 units, ReLU), and an output layer (6 units, softmax activation). The simplified CNN model is shown in Figure 3. The model is compiled with stochastic gradient descent optimization (SGD, learning rate=0.001, momentum=0.9), categorical cross entropy as loss function, categorical accuracy as metrics, 64 batch size, and 20 epochs. A total of 4740 images are used as input for CNN, which requires enormous processing power to analyze images. So, to train our model quickly and effectively in the cloud, we use the Google Colab with NVIDIA Tesla T4 with 12GB of GPU memory and 78GB of disk space. This platform provides us with a high-end CPU and GPU built with all the necessary packages for the training process [Bisong, 2019]. The comparison of the proposed model has been performed with the pre-trained models which include VGG16, ResNet50, and InceptionV3 (include_top =False, weights= imagenet). All of the models were trained and tested on the same dataset with an 80-20% split. A standard evaluation method, 5-fold cross-validation approach is used to assess the robustness of our proposed model.

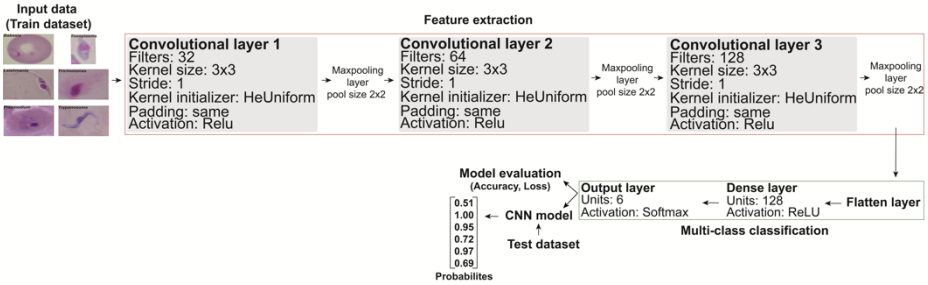

Figure 3: The proposed three convolutional layers of the CNN model

3.4 Experimental Setup

Our model is evaluated using a train and test methodology. The previously mentioned 4740 microscopic images were divided into two subsets, with 80% (3792 images) used to train the classification network and 20% (948 images) used to test the model's overall performance. The performance of test data for the proposed model is assessed using the following metrics: accuracy, loss, confusion matrix, and classification report (precision, recall, F1 score), and accuracy and classification report are used for pre-trained models.

Accuracy: It is defined as the ratio of the sum of true positive (TP) and true negative (TP) values to the sum of TP, TN, FP, and FN values. The mathematical expression for accuracy is shown in equation (1)

$$\text{Accuracy} = \frac{TP + TN}{TN + TP + FP + FN} \quad (1)$$

Categorical cross-entropy loss: It measures the difference between the predicted probability distribution and the actual probability distribution. The mathematical expression for the cross-entropy loss function is shown in equation (2) [Yathish, 2022].

$$CE\ Loss = -\frac{1}{n}\sum_{i=1}^{N}\sum_{j=1}^{M} y_{ij} \cdot log(p_{ij}) \quad (2)$$

Precision: It can be also called positive predictive value. It is defined as the ratio of the correctly predicted positive observations (TP) of the total predicted positive observations (TP, FP). The mathematical expression for precision is shown in equation (3)

$$\text{Precision} = \frac{\text{TP}}{\text{TP} + \text{FP}} \quad (3)$$

Recall: It can be also called sensitivity or true positive rate (TPR). It is the ratio of correctly predicted positive observations to all observations in an actual class. The mathematical expression for the recall is shown in equation (4)

$$\text{Recall} = \frac{\text{TP}}{\text{TP} + \text{FN}} \quad (4)$$

F1 score: It is the weighted average of precision and recall. Thus it takes both false positives and false negatives into account. The mathematical expression for F1score is shown in equation (5)

$$\text{F1 score} = 2 \text{ x } \frac{\text{Recall x Precision}}{\text{Recall + Precision}} \quad (5)$$

4 Results

4.1 Evaluation of the Model Performance

We plotted the loss and accuracy curve of the train and test datasets after 20 epochs to determine the performance of the CNN model, and the testing accuracy is 94.20%. The loss and accuracy curves for the train and test datasets are both relatively smooth, with just minor variations. Furthermore, the train and test loss is continuously decreasing to a value close to 0.25, indicating that the model fits the problem well (Figure 4). The performance evaluation in all five folds shows a range of accuracy (95.60%-97.59%), precision range (of 0.91-1), recall range (of 0.89-1), and F1-score range (of 0.92-1) respectively (Table 2).

	Number of folds				
	1	2	3	4	5
Accuracy (%)	97.59	95.88	95.60	97.16	97.16
Precision	0.93-1	0.93-98	0.91-0.99	0.92-1	0.94-1
Recall	0.94-1	0.89-99	0.91-1	0.95-1	0.96-0.98
F1-score	0.95-1	0.92-99	0.94-1	0.95-1	0.95-0.99

Table 2: Detail analysis of five-fold cross-validation

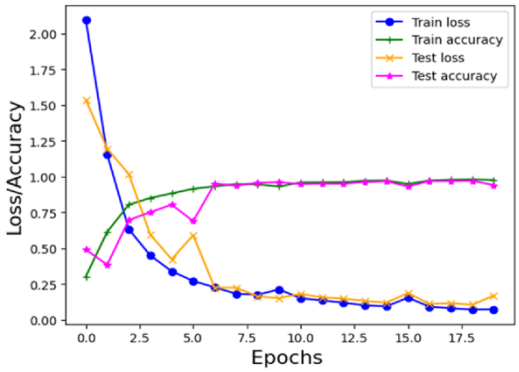

Figure 4: Train loss and accuracy versus test loss and accuracy

4.2 Performance Evaluation using Test Data

Out of 948 test data results, 893 images, or more than 120 images for each class (Figure 5A), were accurately predicted across all six classes, with an accuracy rate of more than 94% (Figure 5B) except for class *Leishmania* (76%). *Leishmania* had the highest misclassification rate of all the six classes, with 38 images (out of 158 images, 24%) mistakenly identified as *Plasmodium* and *Trichomonas* (Figure 5A) with an accuracy of 16% and 7.6% respectively (Figure 5B).

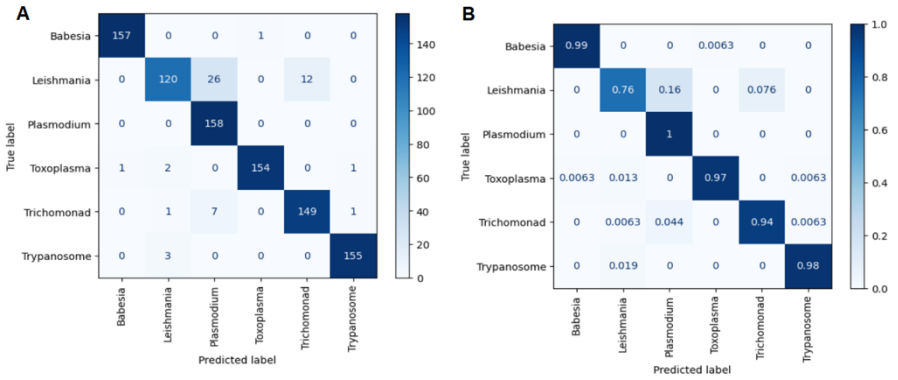

Figure 5: The confusion matrix on the multi-class test data displays the number and proportion of correctly and incorrectly predicted values. A) confusion matrix without normalization, B) confusion matrix with normalization

Figure 6A shows the correct identification of all classes with an accuracy of above 95%, whereas Figure 6B shows the incorrect identification of five classes. The sample image of *Babesia* is displayed as *Toxoplasma,* with an accuracy possibility of 84.98%. The sample images of *Leishmania* are displayed as *Plasmodium* (90%) and *Trichomonas* (72%), respectively. The sample image of *Toxoplasma* is displayed as *Babesia* (92%), *Leishmania* (53%), and *Trypanosoma* (97%), respectively. The sample image of *Trichomonas* is displayed as *Leishmania* (51%) and *Plasmodium* (91%) respectively and finally, the sample image of *Trypanosoma* is displayed as *Leishmania* with an accuracy possibility of 69%. The proposed model classification report for each class in the test dataset is provided in Table 3, and the comparison to pre-trained models is shown in Table 4. Additionally, despite the high accuracy of two models, ResNet50 (97.26%) and InceptionV3 (99.05%), it is observed that the train and test loss (results not provided) do not consistently decrease as shown in Figure 4. This suggests that the models (ResNet50, InceptionV3) did not adequately fit the data.

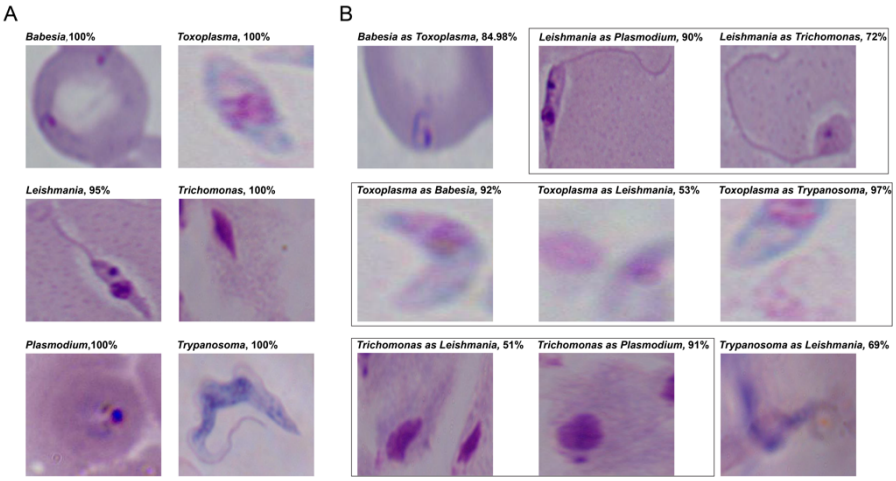

Figure 6: Some outcomes of correctly and incorrectly identified test data images from six classes. A) correctly identified test data images, B) incorrectly identified test data images

	Precision	**Recall**	**F1-score**	**Support**
Babesia	0.99	0.99	0.99	158
Leishmania	0.95	0.76	0.85	158
Plasmodium	0.83	1	0.91	158
Toxoplasma	0.99	0.97	0.98	158
Trichomonas	0.93	0.94	0.93	158
Trypanosoma	0.99	0.98	0.98	158
Accuracy	-	-	0.94	948
Macro avg	0.95	0.94	0.94	948
Weighted avg	0.95	0.94	0.94	948

Table 3: Precision, recall, and F1 score values for six classes

Model	**Accuracy (%)**	**Precision**	**Recall**	**F1-score**
Proposed CNN	94	0.83-0.99	0.76-1	0.85-0.99
VGG16	92.51	0.83-0.98	0.66-1	0.78-0.98
ResNet50	97.26	0.91-1	0.91-1	0.95-0.98
InceptionV3	99.05	0.98-1	0.98-1	0.98-1

Table 4: Results obtained for all models

5 Discussion

This study used three convolutional layers of a CNN with SGD optimizer to achieve the multi-class classification of six protozoan parasites. The model's performance evaluation reveals good accuracy and no overfitting behavior in the plot (Figure 4), showing that the model was effectively trained concerning both datasets. This model enables the accurate identification of most cases with an accuracy of 94% for 20 epochs, a precision range of 0.83-0.99, and a recall range of 0.76-1.00. The robustness of the proposed model showed an accuracy range of 95.60%-97.59%, a precision range of 0.93-1.00, and a recall range of 0.89-1.00 for all five folds. The model's performance evaluation with three layers of CNN used in this study can be comparable to earlier studies. The comparable study with a similar dataset (i.e., protozoan parasites) has been performed with Xception architecture using different optimizers, the results are as follows Adam optimizer (accuracy: 97%, precision range 0.93-1, recall range 0.92-0.99) and SGD optimizer (accuracy: 89%, precision range 0.87-0.93, recall range 0.72-0.98) respectively [Al Maki et al., 2023]. The detection of *Plasmodium* parasites with three convolutional layers using preprocessing steps such as sheer range, zoom range, and horizontal flip with 8000 train images and 2000 test images achieved an accuracy of 95% [Shah et al., 2020]. In contrast to the other studies (Table 6) that used DenseNet121 to study *Babesia*-Infected erythrocytes shows a precision and recall of 0.92 and 1.00 with an accuracy of 99% [Durant et al., 2022], our study revealed a precision and recall of 0.99 and 0.99.

In an experimental murine model, a U-Net CNN architecture was implemented and trained on *T.cruzi* amastigotes on histopathological images obtained from an endomyocardial biopsy showed an accuracy of 99.19% [Sanchez-Patio et al., 2021]. Similarly, in another study, MobileNet V2 convolutional layers were used to detect *T. cruzi* from acute-phase peripheral blood samples with image tiles. On a balanced validation subset, the image tiles from a 12-slide dataset displayed an accuracy of 96.4%. The test accuracy was found to be 72% from 13 blood smear slides, the test accuracy increased to 95.4% when the dataset was expanded [Pereira et al., 2022]. In a different study, on a dataset of 160 eye fundus images, three deep-learning models with data augmentation by random flips and crops were applied. The following DL model's accuracy is as follows VGG16 (96.8%), Resnet18 (93.75%), and Vanilla CNN (75%) [Parra et al., 2021]. *Trichomonas vaginalis* was identified using two CNN with encoder-decoder architecture, and the accuracy was 72.09% [Wang et al., 2021]. In this study, despite having an overall accuracy of 94% compared to other classes, *Leishmania* has the highest percentage of incorrectly identified classes (*Plasmodium* and *Trichomonas*), indicating that the features of this particular sample have not been well learned by the model and may be referred to as adversarial or conjecture examples. This dataset might benefit from adding more convolutional layers or data augmentation parameters.

Datasets	Accuracy (%)	Model	References
Plasmodium	95	CNN	Shah et al., 2020
Babesia-Infected erythrocytes	99	DenseNet121	Durant et al., 2022
T.cruzi amastigotes *T.cruzi from blood smears*	99.19 95.4	U-Net CNN MobileNet V2	Sanchez-Patio et al., 2021 Pereira et al., 2022
Toxoplasma gondii	96.8	VGG16	Parra et al., 2021
Trichomonas vaginalis	72.09	CNN	Wang et al., 2021
Human Protozoan Parasites	97	Xception architecture	Al Maki et al., 2023
Six classes of protozoan parasites	**94**	**CNN**	**Proposed model**

Table 6: Performance comparison between deep learning models on protozoan parasites dataset in literature

6 Conclusion

Our work, presented in this study, demonstrates how effectively the CNN with three convolutional layers can be utilized to detect multi-class classification of the protozoan parasites. However, more work needs to be done to reduce the misclassification of the class *Leishmania* and to build a better deep learning model like a human expert would. In the future, the model will be improved, and it will be combined with a smartphone app to help with real-time parasite identification and categorization.

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Data Availability: The data that support the findings of this study are openly available in the Mendeley data repository at https://data.mendeley.com/datasets/38jtn4nzs6/3.

Author Contributions: S.E: Reviewing and Editing. S.Y: Reviewing and Editing. V.S: Reviewing and Editing. S.N: Conceptualization, Methodology, Visualization, Investigation, Writing – Original draft preparation.

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